PCT

REQUEST

Rec'd PCT/PTO 12 OCT 199

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference: *(if desired) (12 characters maximum)* **CPI-042CPPC**

Box No. I TITLE OF INVENTION

MEKK1 PROTEINS AND FRAGMENTS THEREOF FOR USE IN REGULATING APOPTOSIS

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

CADUS PHARMACEUTICAL CORPORATION
777 Old Saw Mill River Road
Tarrytown, New York 10591
United States of America

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(i.e. country)* of nationality: **US**

State *(i.e. country)* of residence: **US**

This person is applicant for the purposes of : ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

JOHNSON, Gary L.
2497 Keller Farm Drive
Boulder, Colorado 80304
United States of America

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: **US**

State *(i.e. country)* of residence: **US**

This person is applicant for the purposes of : ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

DECONTI, Giulio A., Jr.
Lahive & Cockfield, LLP
28 State Street
Boston, Massachusetts 02109
United States of America

Telephone No. **(617) 227-7400**

Facsimile No. **(617) 742-4214**

Teleprinter No. **N/A**

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

☒ **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)*.....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒	**AL**	Albania	☒	**LS**	Lesotho .....
☒	**AM**	Armenia	☒	**LT**	Lithuania
☒	**AT**	Austria	☒	**LU**	Luxembourg .....
☒	**AU**	Australia	☒	**LV**	Latvia
☒	**AZ**	Azerbaijan	☒	**MD**	Republic of Moldova .....
☒	**BA**	Bosnia and Herzegovina	☒	**MG**	Madagascar .....
☒	**BB**	Barbados	☒	**MK**	The former Yugoslav Republic of Macedonia ...
☒	**BG**	Bulgaria			.....
☒	**BR**	Brazil	☒	**MN**	Mongolia
☒	**BY**	Belarus	☒	**MW**	Malawi .....
☒	**CA**	Canada	☒	**MX**	Mexico .....
☒	**CH and LI**	Switzerland and Liechtenstein	☒	**NO**	Norway
☒	**CN**	China	☒	**NZ**	New Zealand .....
☒	**CU**	Cuba	☒	**PL**	Poland .....
☒	**CZ**	Czech Republic	☒	**PT**	Portugal .....
☒	**DE**	Germany	☒	**RO**	Romania
☒	**DK**	Denmark	☒	**RU**	Russian Federation .....
☒	**EE**	Estonia	☒	**SD**	Sudan
☒	**ES**	Spain	☒	**SE**	Sweden
☒	**FI**	Finland	☒	**SG**	Singapore
☒	**GB**	United Kingdom	☒	**SI**	Slovenia .....
☒	**GD**	Grenada	☒	**SK**	Slovakia .....
☒	**GE**	Georgia	☒	**SL**	Sierra Leone
☒	**GH**	Ghana	☒	**TJ**	Tajikistan .....
☒	**GM**	Gambia	☒	**TM**	Turkmenistan .....
☒	**HR**	Croatia	☒	**TR**	Turkey .....
☒	**HU**	Hungary	☒	**TT**	Trinidad and Tobago .....
☒	**ID**	Indonesia	☒	**UA**	Ukraine .....
☒	**IL**	Israel	☒	**UG**	Uganda .....
☒	**IN**	India	☒	**US**	United States of America .....
☒	**IS**	Iceland			.....
☒	**JP**	Japan	☒	**UZ**	Uzbekistan .....
☒	**KE**	Kenya	☒	**VN**	Viet Nam
☒	**KG**	Kyrgyzstan	☒	**YU**	Yugoslavia
☒	**KP**	Democratic People's Republic of Korea	☒	**ZW**	Zimbabwe .....
☒	**KR**	Republic of Korea			
☒	**KZ**	Kazakstan			
☒	**LC**	Saint Lucia			
☒	**LK**	Sri Lanka			
☒	**LR**	Liberia			

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

☐ .....

☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) **13 Feb 1998 (13.02.98)**	**09/023,130**	**US**		
item (2)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): **(1)**

**Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA/EP

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* | Number | Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets:**

request	:	4
description (excluding sequence listing part)	:	71
claims	:	10
abstract	:	1
drawings	:	25
sequence listing part of description	:	41
Total number of sheets	:	152

This international application is **accompanied by** the item(s) marked below:

1. ☒ **fee calculation sheet**
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: **Transmittal Letter and Return Postcard.**

Figure of the drawings which should accompany the abstract: **NONE**

Language of filing of the international application: **ENGLISH**

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

For CADUS PHARMACEUTICAL CORPORATION et al.

Peter C. Lauro

Peter C. Lauro

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	☐ received:
4. Date of timely receipt of the required corrections under PCT Article 11(2):	☐ not received:
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

Supplemental Box *If the Sup[illegible]ox is not used, this sheet need not be u[illegible] the request.*

Continuation of Box IV

Agent:

LIEPMANN, W. Hugo
COCKFIELD, James E.
SMURZYNSKI, Thomas V.
LOREN, Ralph A.
LYNCH, Jeremiah
HAMMITTE, Ann Lamport
HANLEY, Elizabeth A.
MANDRAGOURAS, Amy E.
BIANCO, John V.
LAURENTANO, Anthony A.
REMILLARD, Jane E.
CANNING, Kevin J.
MONKS, Lawrence E.
LANE, Jr., David A.
KARA, Catherine J.
CHINN, Linda M.
LAURO, Peter C.
LICHAUCO, Faustino A.
DiGIORGIO, Jeanne M.
WILLIAMS, Megan E.
TRIANO III, Nicholas P.
WELCH, John L.
MOLLAAGHABABA, Reza
DOUROS, Timothy J.

The above attorneys and agents are members of the firm of Lahive & Cockfield, LLP. Address, telephone number, facsimile number and teleprinter number of all are indicated in Box IV.

Continuation of Box V.

United States of America

Application No.: 09/023,130
Filed 13 February 1998 (13.02.98)

09/403,075

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/54, 9/12, 5/10, 15/62, C07K 19/00, 16/40, G01N 33/573, C12Q 1/68, A61K 31/00, 48/00

A1

(11) International Publication Number: WO 99/41385

(43) International Publication Date: 19 August 1999 (19.08.99)

(21) International Application Number: PCT/US99/02974

(22) International Filing Date: 12 February 1999 (12.02.99)

(30) Priority Data:
09/023,130 13 February 1998 (13.02.98) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 09/023,130 (CON)
Filed on 13 February 1998 (13.02.98)

(71) Applicant *(for all designated States except US)*: CADUS PHARMACEUTICAL CORPORATION [US/US]; 777 Old Saw Mill River Road, Tarrytown, NY 10591 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: JOHNSON, Gary, L. [US/US]; 2497 Keller Farm Drive, Boulder, CO 80304 (US).

(74) Agents: DECONTI, Giulio, A., Jr. et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US).

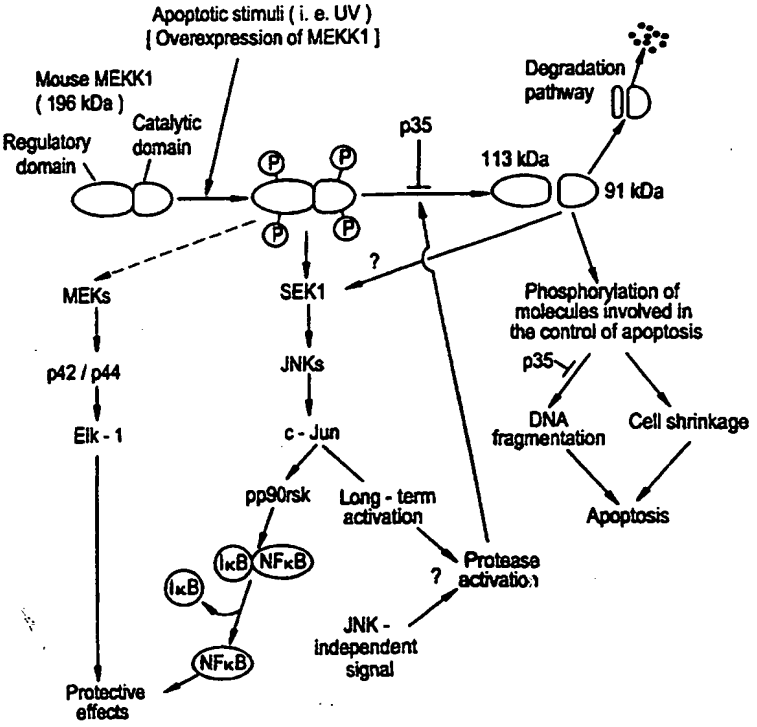
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: MEKK1 PROTEINS AND FRAGMENTS THEREOF FOR USE IN REGULATING APOPTOSIS

(57) Abstract

The present invention relates to isolated MEKK1 proteins, nucleic acid molecules having sequences that encode such proteins, and antibodies raised against such proteins. The present invention also includes methods to use such proteins to regulate apoptosis. The invention provides active fragments of MEKK1 proteins that are generated upon cleavage of MEKK1 with a caspase protease. These active fragments are capable of stimulating apoptosis. Moreover, the invention provides protease–resistant forms of MEKK1 proteins, that are resistant to cleavage by caspase proteases and that are capable of inhibiting apoptosis. Still further, the invention provides methods for generating an active fragment of MEKK1, methods of identifying modulators of the apoptotic activity of an active fragment of MEKK1 and methods of identifying modulators of caspase–mediated cleavage of MEKK1.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

MEKK1 PROTEINS AND FRAGMENTS THEREOF FOR USE IN REGULATING APOPTOSIS

Government Funding

This invention was made in part with government support under USPHS Grants DK37871, DK48845, CA58157 and GM30324, each awarded by the National Institutes of Health. The government has certain rights in this invention.

Field of the Invention

This invention relates to isolated nucleic acid molecules encoding MEKK1 proteins, substantially pure MEKK1 proteins, and products and methods for regulating apoptosis in cells.

Background of the Invention

Mitogen-activated protein kinase (MAPKs) (Mitogen-Activated Protein Kinases, also called extracellular signal-regulated kinases or ERKs) are rapidly activated in response to ligand binding by both growth factor receptors that are tyrosine kinases and receptors that are coupled to heterotrimeric guanine nucleotide binding proteins (G proteins). MAPKs integrate multiple intracellular signals transmitted by various second messengers *via* a mechanism which involves the phosphorylation and regulation of the activity of enzymes and transcription factors including the EGF receptor, Rsk 90, phospholipase A_2, c-Myc, c-Jun and Elk-1/TCF.

MAPKs are in turn phosphorylated and regulated by proteins called MEKs (MAPK Kinase or ERK Kinase) or MKK (MAP Kinase kinase). The MEKs phosphorylate MAPKs on both tyrosine and threonine residues which results in activation of MAPKs. MEKs are likewise phosphorylated and regulated by one of two distinct classes of mammalian serine-threonine protein kinase, the Rafs or the MEKKs (MEK Kinases).

Certain biological functions, such as growth and differentiation, are tightly regulated by signal transduction pathways within cells. Signal transduction pathways maintain the balanced steady state functioning of a cell. Disease states can arise when signal transduction in a cell breaks down, thereby removing the tight control that typically exists over cellular functions. For example, tumors develop when regulation of cell growth is disrupted, enabling a clone of cells to expand indefinitely. Because signal transduction networks regulate a multitude of cellular functions depending upon the cell type, a wide variety of diseases can result from abnormalities in such networks. Devastating diseases such as cancer, autoimmune diseases, allergic reactions,

inflammation, neurological disorders and hormone-related diseases can result from abnormal signal transduction.

Given the importance of signal transduction molecules in regulating a variety of cellular processes and the important consequences of signal transduction aberrancies in disease states, there exists a need to identify novel signaling molecules. Moreover, understanding intracellular signaling pathways is advantageous in identifying and developing pharmacological and therapeutic agents targeted towards particular signaling molecules.

Summary of the Invention

The present invention is based, at least in part, on the identification of MEKK1 protein and nucleic acid molecules, in particular, human MEKK1 molecules, as well as bioactive fragments of MEKK1 molecules useful in regulating cellular apoptosis.

In one aspect, the present invention relates to isolated nucleic acid molecules having sequences that encode MEKK1 proteins, MEKK1 proteins, and antibodies raised against such proteins. In one embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:3. The sequence of SEQ ID NO:3 corresponds to a murine MEKK1 cDNA. The predicted amino acid sequence of murine MEKK1 is set forth as SEQ ID NO 4. This cDNA comprises sequences encoding the murine MEKK1 protein (*i.e.*, "the coding region", from nucleotides 15-4496), as well as 5' untranslated sequences (nucleotides 1-14) and 3' untranslated sequences (nucleotides 4497-5253). The predicted amino acid sequence of murine MEKK1 is set forth as SEQ ID NO 4. In another embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:5. The sequence of SEQ ID NO:5 corresponds to a human MEKK1 cDNA. This cDNA comprises sequences encoding human MEKK1 protein (*i.e.*, a "coding region", from nucleotides 3-3911). The predicted amino acid sequence of human MEKK1 is set forth as SEQ ID NO 5.

In another aspect, this invention provides isolated nucleic acid molecules encoding MEKK1 proteins or biologically active portions or fragments thereof (*e.g.*, apoptotic portions or fragments), as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of MEKK1-encoding nucleic acids.

In one embodiment, a MEKK1 nucleic acid molecule is 90% homologous to the nucleotide sequence shown in SEQ ID NO:3, SEQ ID NO:5, or complement thereof. In a preferred embodiment, an isolated MEKK nucleic acid molecule has the nucleotide sequence shown SEQ ID NO:3, or a complement thereof. In another embodiment, a MEKK nucleic acid molecule comprises nucleotides 15-4496 of SEQ ID NO:3. In

another preferred embodiment, an isolated MEKK nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:5. In another embodiment, a MEKK1 nucleic acid molecule comprises nucleotides 3-3911 of SEQ ID NO:5.

In another embodiment, a MEKK1 nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence substantially homologous to the amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6. In another preferred embodiment, a MEKK1 nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 90% homologous to the amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6. In yet another embodiment, a MEKK1 nucleic acid molecule is a naturally occurring nucleotide sequence (*e.g.*, a naturally-occurring human or murine nucleotide sequence).

Another embodiment of the invention features isolated nucleic acid molecules which specifically detect MEKK1 nucleic acid molecules relative to nucleic acid molecules encoding non-MEKK1 proteins. For example, in one embodiment, an isolated nucleic acid molecule hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:3, SEQ ID NO:5, or a complement thereof. In another embodiment, an isolated nucleic acid molecule hybridizes to about nucleotides 1-2400 of SEQ ID NO:3. In another embodiment, an isolated nucleic acid molecule is at least 500 nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:3, SEQ ID NO:5, or a complement thereof

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to the coding strand of a MEKK1 nucleic acid.

Another aspect of the invention provides a vector comprising a MEKK1 nucleic acid molecule. In certain embodiments, the vector is a recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. The invention also provides a method for producing a MEKK1 protein by culturing in a suitable medium, a host cell of the invention containing a recombinant expression vector such that a MEKK1 protein is produced.

Another aspect of this invention features isolated or recombinant MEKK1 proteins and polypeptides. In one embodiment, an isolated protein includes a biologically active portion of a MEKK1 protein (*e.g.*, an apoptotic portion). In another embodiment, an isolated MEKK1 protein has an amino acid sequence substantially homologous to the amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6. In a preferred embodiment, a MEKK1 protein has an amino acid sequence at least about 90% homologous to the amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6. In another

embodiment, a MEKK1 protein has the amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6.

Another embodiment of the invention features an isolated MEKK1 protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 90% homologous to a nucleotide sequence of SEQ ID NO:3, SEQ ID NO:5, or a complement thereof. This invention further features an isolated MEKK1 protein which is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:3, SEQ ID NO:5, or a complement thereof.

The MEKK1 proteins of the present invention, or biologically active portions thereof, can be operatively linked to a non-MEKK1 polypeptide (*e.g.*, heterologous amino acid sequences) to form MEKK1 fusion proteins. The invention further features antibodies that specifically bind MEKK1 proteins, such as monoclonal or polyclonal antibodies. In addition, the MEKK1 proteins or biologically active portions thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting the presence of a MEKK1 protein in a sample (*e.g.*, biological sample) by contacting the sample with a compound which selectively binds to the protein and determining whether the compound binds to the protein in the sample to thereby detect the presence of a MEKK1 protein in the sample.

In another aspect, the present invention provides a method for detecting the presence of a MEKK1 nucleic acid molecule in a sample (*e.g.*, biological sample) by contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule and determining whether the probe or primer binds to a nucleic acid molecule in the sample to thereby detect the presence of a MEKK1 nucleic acid molecule in the sample.

In another aspect, the present invention provides a method for detecting the presence of MEKK1 activity in a biological sample by contacting the biological sample with an agent capable of detecting MEKK1 activity such that the presence of MEKK1 activity is detected in the biological sample.

In another aspect, the invention provides a method for modulating MEKK1 activity comprising contacting the cell with an agent that modulates MEKK1 activity such that MEKK1 activity in the cell is modulated. In one embodiment, the agent inhibits MEKK1 activity. In another embodiment, the agent stimulates MEKK1 activity. In one embodiment, the agent is an antibody that specifically binds to a MEKK1 protein. In another embodiment, the agent modulates expression of MEKK1

by modulating transcription of a MEKK1 gene or translation of a MEKK1 mRNA. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of a MEKK1 mRNA or a MEKK1 gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant MEKK1 protein or nucleic acid expression or activity by administering an agent which is a MEKK1 modulator to the subject. In one embodiment, the MEKK1 modulator is a MEKK1 protein. In another embodiment the MEKK1 modulator is a MEKK1 nucleic acid molecule. In yet another embodiment, the MEKK1 modulator is a peptide, peptidomimetic, or other small molecule. In a preferred embodiment, the disorder characterized by aberrant MEKK1 protein or nucleic acid expression is a developmental, differentiative, proliferative disorder, an immunological disorder, or cell death.

The present invention also provides a diagnostic assay for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a MEKK1 protein; (ii) mis-regulation of said gene; and (iii) aberrant post-translational modification of a MEKK1 protein, wherein a wild-type form of said gene encodes an protein with a MEKK1 activity.

The present invention also includes methods to use MEKK1 proteins to regulate apoptosis. The invention provides active fragments of MEKK1 proteins that are generated upon cleavage of MEKK1 with a caspase protease. These active fragments are capable of stimulating apoptosis. Moreover, the invention provides protease-resistant forms of MEKK1 proteins, that are resistant to cleavage by caspase proteases and that are capable of inhibiting apoptosis. Still further, the invention provides methods for generating an active fragment of MEKK1, methods of identifying modulators of the apoptotic activity of an active fragment of MEKK1 and methods of identifying modulators of caspase-mediated cleavage of MEKK1.

It has been discovered that MEK kinase 1 (MEKK1), a 196 kDa protein kinase, functions to integrate proteases and signal transduction pathways involved in the regulation of apoptosis. Cleavage of mouse MEKK1 at Asp^{874} generates a 91 kDa kinase fragment and a 113 kDa NH_2-terminal fragment. The kinase fragment of MEKK1 induces apoptosis. Cleavage of MEKK1 and apoptosis are inhibited by p35 and CrmA, viral inhibitors of the ICE/FLICE proteases that commit cells to apoptosis. Mutation of the MEKK1 sequence $^{871}DTVD^{874}$, a cleavage site for CCP32-like proteases, to alanines inhibited proteolysis of MEKK1 and apoptosis induced by overexpression of MEKK1. Inhibition of MEKK1 proteolysis inhibited apoptosis but did not block MEKK1 stimulation of c-Jun kinase activity, indicating that c-Jun kinase activation was not sufficient for apoptosis. During the apoptotic response to UV

irradiation, cisplatin, etoposide and mitomycin C, MEKK1 undergoes a phosphorylation-dependent activation followed by its proteolysis. These results show that MEKK1 activation and cleavage occurs in response to genotoxic agents and the activated kinase fragment functions to commit cells to apoptosis.

Accordingly, this invention defines MEKK1 as a protease substrate that when activated and cleaved stimulates an apoptotic response. The proteolytic cleavage of MEKK1 defines the mechanism to generate a protein kinase whose activity is sufficient to induce apoptosis. In the context of cancer therapy, the finding that the activation and cleavage of MEKK1 occurs in response to genotoxic agents is particularly important. It has been found that expression of MEKK1 is capable of killing by apoptosis cells that have both p53 alleles mutated. Hence, the activation and cleavage of MEKK1 is an apoptotic pathway that does not require a functional p53 and stimulation of these events could enhance the killing of many different tumors. Manipulating the activation of MEKK1 and its cleavage by proteases, with the use of drugs for example, could increase the killing of tumor cells to genotoxic agents. Consistent with this hypothesis is the finding that low level expression of MEKK1 potentiated the apoptotic response to low doses of UV irradiation and cisplatin.

One aspect of the present invention pertains to active fragments of MEKK1 proteins (*i.e.*, fragments of MEKK1 proteins that retain apoptotic activity). Such active fragments can be generated naturally by cleavage of MEKK1 by a caspase protease. For example, an apoptotic fragment of murine MEKK1 can be generated by caspase after a cleavage site found at amino acids 871-874 of SEQ ID NO:4. Likewise, an apoptotic fragment of human MEKK1 can be generated by caspase after a cleavage site found at amino acids 681-684 of SEQ ID NO:6. Alternatively, the active fragments of the invention can be prepared by recombinant DNA technology, using standard methodologies. In one embodiment, the invention provides an isolated active fragment of an MEKK1 protein consisting of an amino acid sequence having at least 75% homology to an amino acid sequence consisting of about amino acids 875-1493 of SEQ ID NO:4, wherein said active fragment mediates apoptosis. Preferably, the active fragment consists of an amino acid sequence having at least 85% homology to an amino acid sequence consisting of about amino acids 875-1493 of SEQ ID NO:4. More preferably, the active fragment consists of an amino acid sequence having at least 95% homology to an amino acid sequence consisting of about amino acids 875-1493 of SEQ ID NO:4. In one embodiment, the active fragment is a mouse MEKK1 active fragment. In another embodiment, the active fragment is a human MEKK1 active fragment. In another embodiment, the active fragment is a rat MEKK1 active fragment. The active fragment can consist of, for example, about amino acids 875-1493 of SEQ ID NO:4.

Preferably, the active fragment consists of amino acids 875-1493 of SEQ ID NO:4. The active fragment can consist of about amino acids 685-1303 of SEQ ID NO:6. Preferably, the active fragment consists of amino acids 685-1303 of SEQ ID NO:4.

Another aspect of the invention pertains to protease-resistant forms of MEKK1 proteins. Such protease-resistant forms can be generated by mutation of the caspase cleavage site in an MEKK1 protein (*e.g.*, a cleavage site corresponding to amino acids 871-874 of SEQ ID NO:4 or amino acids 681-684 of SEQ ID NO:6) such that the site cannot be cleaved by the caspase. Preferably, at least the Asp residue at 871 and/or 874 of SEQ ID NO:4 is mutated. Alternatively, at least the Asp residue at 681 and/or 684 of SEQ ID NO:6 is mutated. Preferably, one or more of the amino acids corresponding to 871-874 of SEQ ID NO:4 or to 681-684 of SEQ ID NO:6 can be mutated to, for example, alanine residues. Alternatively, said residue can be mutated to glutamine. Accordingly, the invention provides an isolated protease-resistant MEKK1 protein comprising an amino acid sequence having at least 75% homology to the amino acid sequence of SEQ ID NO:4, wherein at least one amino acid equivalent to amino acids 871-874 of SEQ ID NO:4 is substituted such that the MEKK1 protein is resistant to proteolysis by a caspase. Preferably, the protease-resistant MEKK1 protein has at least 85% homology to the amino acid sequence of SEQ ID NO:4. More preferably, the protease-resistant MEKK1 protein has at least 95% homology to the amino acid sequence of SEQ ID NO:4. In one embodiment, the protease-resistant MEKK1 protein is a mouse MEKK1 protein. In another embodiment, the protease-resistant MEKK1 protein is a human MEKK1 protein. In yet another embodiment, the protease-resistant MEKK1 protein is a rat MEKK1 protein.

The invention further provides isolated nucleic acid molecules that encode the MEKK1 active fragments of the invention. In one embodiment, the invention provides an isolated nucleic acid molecule consisting of a nucleotide sequence having at least 75% homology to a nucleotide sequence consisting of about nucleotides 2637-4493 of SEQ ID NO:3, wherein said nucleic acid molecule encodes an active fragment of MEKK1 that mediates apoptosis. Preferably, the nucleic acid molecule consists of a nucleotide sequence having at least 85% homology to a nucleotide sequence consisting of about nucleotides 2637-4493 of SEQ ID NO:3. More preferably, the nucleic acid molecule consists of a nucleotide sequence having at least 95% homology to a nucleotide sequence consisting of about nucleotides 2637-4493 of SEQ ID NO:3. In one embodiment, the nucleic acid molecule encodes an active fragment of mouse MEKK1. In another embodiment, the nucleic acid molecule encodes an active fragment of human MEKK1. In yet another embodiment, the nucleic acid molecule encodes an active fragment of rat MEKK1. In a preferred embodiment, the nucleic acid molecule

comprises at least about nucleotides 2637-4493 of SEQ ID NO:3, or a nucleotide sequence that, due to the degeneracy of the genetic code, encodes the same amino acid sequence as about nucleotides 2637-4493 of SEQ ID NO:3. In another preferred embodiment, the nucleic acid molecule comprises at least about nucleotides 2052-3908 of SEQ ID NO:5, or a nucleotide sequence that, due to the degeneracy of the genetic code, encodes the same amino acid sequence as nucleotides 2052-3908 of SEQ ID NO:5.

The invention also provides isolated nucleic acid molecules encoding the protease-resistant forms of MEKK1 of the invention. For example, the invention provides an isolated nucleic acid molecule encoding a protease-resistant MEKK1 protein, wherein the protease resistant MEKK1 protein comprises an amino acid sequence having at least 75% homology to the amino acid sequence of SEQ ID NO:4 and at least one codon of the nucleic acid molecule encoding an amino acid equivalent to at least one of amino acids 871-874 of SEQ ID NO:4 is mutated such the encoded MEKK1 protein is resistant to proteolysis by a caspase after an amino acid equivalent to amino acid 874 of SEQ ID NO:4. Preferably, the MEKK1 protein comprises an amino acid sequence having at least 85% homology to the amino acid sequence of SEQ ID NO:4. More preferably, the MEKK1 protein comprises an amino acid sequence having at least 95% homology to the amino acid sequence of SEQ ID NO:4. In one embodiment, the nucleic acid encodes a protease-resistant mouse MEKK1 protein. In another embodiment, the nucleic acid encodes a protease-resistant human MEKK1 protein. In yet another embodiment, the nucleic acid molecule encodes a protease-resistant rat MEKK1 protein. In a preferred embodiment, the nucleic acid has the nucleic acid sequence of SEQ ID NO:5 where at least one codon encoding one of amino acids 681-684 of SEQ ID NO:6 is mutated such the encoded MEKK1 protein is resistant to proteolysis by a caspase after an amino acid equivalent to amino acid 684 of SEQ ID NO:6.

Yet another aspect of the invention pertains to methods for modulating apoptosis. In one embodiment, the invention provides a method of stimulating apoptosis in a cell comprising introducing into the cell an expression vector encoding an MEKK1 active fragment of the invention such that MEKK1 active fragment is produced in the cell and apoptosis is stimulated. In another embodiment, the invention provides a method of inhibiting apoptosis in a cell comprising introducing into the cell an expression vector encoding a protease-resistant MEKK1 protein of the invention such that protease-resistant MEKK1 protein is produced in the cell and apoptosis is inhibited.

The invention also provides methods for generating MEKK1 active fragments *in vitro*. For example, an MEKK1 active fragment can be generated *in vitro* by:

contacting an MEKK1 protein *in vitro* with a caspase protease under proteolysis conditions; and

allowing the caspase protease to cleave the MEKK1 protein such that an MEKK1 active fragment is generated.

Preferably, the caspase protease is a caspase-3 protease. Alternatively, the caspase protease is a caspase-7 protease. Standard proteolysis conditions known in the art under which caspase proteases are known to be active can be used in the method of the invention.

Still another aspect of the invention pertains to methods for identifying modulators of apoptosis. In one embodiment, the invention provides a method of identifying a compound that modulates the apoptotic activity of an MEKK1 active fragment. The method comprises:

providing an indicator cell that comprises an MEKK1 active fragment of the invention;

contacting the indicator cell with a test compound; and

determining the effect of the test compound on the apoptotic activity of the MEKK1 active fragment in the indicator cell to thereby identify a compound that modulates the apoptotic activity of the MEKK1 active fragment.

The indicator cell may naturally express an MEKK1 active fragment or may be transfected with an expression vector that encodes the MEKK1 active fragment such that the active fragment is expressed in the cell. The effect of the test compound can be evaluated, for example, by measuring an apoptotic response in the cells, such as DNA fragmentation.

In another embodiment, the invention provides a method of identifying a compound that modulates the proteolytic cleavage of an MEKK1 protein by a caspase protease, comprising:

providing a reaction mixture that comprises an MEKK1 protein and a caspase protease;

contacting the reaction mixture with a test compound; and

determining the effect of the test compound on proteolytic cleavage of the MEKK1 protein by the caspase protease to thereby identify a compound that modulates the proteolytic cleavage of an MEKK1 protein by a caspase protease.

Preferably, the caspase protease is a caspase-3 protease. Alternatively, the caspase protease is a caspase-7 protease. Standard proteolysis conditions known in the art under which caspase proteases are known to be active can be used in the method of the invention. The effect of the test compound on the proteolytic cleavage of MEKK1 can be evaluated by, for example, monitoring the generation of the 91 kD active

fragment of MEKK1 (*e.g.*, by detection of the 91 kD fragment using an anti-MEKK1 antibody, using standard techniques).

Brief Description of the Figures

Figure 1 depicts the cDNA sequence of human MEKK1. The nucleotide sequence corresponds to nucleic acids 1 to 3911 of SEQ ID NO:5.

Figure 2 depicts the cDNA sequence of murine MEKK1. The nucleotide sequence corresponds to nucleic acids 1 to 5253 of SEQ ID NO:3.

Figure 3 depicts an alignment of the amino acid sequences of murine MEKK1 (amino acids 1-1493 of SEQ ID NO:4 and human MEKK1 (amino acids 1-1303 of SEQ ID NO:6). The conserved caspase cleavage site is boxed. Amino acids which are unique as between murine and human MEKK1 are underlined.

Figure 4 is a schematic representation of the HA-tagged mouse MEKK1 protein showing the regions (the numbers correspond to the position of the amino acids) used to generate the indicated antibodies. Also shown is the sequence (one letter code) between amino acids 853 and 888 of SEQ ID NO:4 where the tetrapeptides DEVE (SEQ ID NO: 7) and DTVD (SEQ ID NO: 8) (in bold) have been replaced with alanine residues in mutants DEVE→A and DTVD→A, respectively.

Figure 5 is a schematic representation of the p35-inhibitable and p35-insensitive cleavage in the mouse MEKK1 protein. The letters A to D indicate the names of the cleavage products. The molecular weights were calculated from the migration of the markers in at least 2 different experiments.

Figure 6 is a schematic diagram of a mechanistic model of MEKK1-induced apoptosis.

Figure 7 depicts an alignment of the amino acid sequences of murine MEKK1 and rat MEKK1 (having Accession No. Q62925). The rat MEKK1 amino acid sequence is set forth as SEQ ID NO:21. The predicted caspase cleavage site in rat is boxed. A predicted rat apoptotic fragment begins after the cleavage site and comprises amino acid residues 875-1493 of SEQ ID NO:21.

Figure 8 depicts an alignment of the amino acid sequences of murine MEKK1, rat MEKK1 and partial amino acid sequences of human MEKK1.

Detailed Description of the Invention

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example,

Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); *DNA Cloning*, Volumes I and II (D. N. Glover ed., 1985); *Oligonucleotide Synthesis* (M. J. Gait ed., 1984); Mullis *et al.* U.S. Patent No: 4,683,195; *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins eds. 1984); *Transcription And Translation* (B. D. Hames & S. J. Higgins eds. 1984); *Culture Of Animal Cells* (R. I. Freshney, Alan R. Liss, Inc., 1987); *Immobilized Cells And Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide To Molecular Cloning* (1984); the treatise, *Methods In Enzymology* (Academic Press, Inc., N.Y.); *Gene Transfer Vectors For Mammalian Cells* (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); *Methods In Enzymology*, Vols. 154 and 155 (Wu *et al.* eds.), *Immunochemical Methods In Cell And Molecular Biology* (Mayer and Walker, eds., Academic Press, London, 1987); *Handbook Of Experimental Immunology*, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

The present invention concerns the discovery of novel mitogen ERK kinase kinase proteins (referred to herein as "MEK kinases", "MEKKs" or "MEKK proteins") which function in intracellular signal transduction pathways in a variety of cells, and accordingly have a role in determining cell/tissue fate and maintenance. A salient feature of the MEKK1 gene product is the discovery of the involvement of MEKK1 proteins in certain apoptotic pathways.

Accordingly, certain aspects of the present invention relate to nucleic acids encoding vertebrate MEKK1 proteins (*e.g.*, human and murine MEKK1 proteins), the MEKK1 proteins themselves, antibodies immunoreactive with MEKK1 proteins, and preparations of such compositions. Moreover, the present invention provides diagnostic and therapeutic assays and reagents for detecting and treating disorders involving, for example, aberrant expression or activation of the MEKK1 gene products. In addition, drug discovery assays are provided for identifying agents which can modulate the biological function of MEKK1 proteins, such as by altering the binding of the protein to either downstream or upstream elements in a signal transduction pathway, or which inhibit the kinase activity of the MEKK1 protein. Such agents can be useful therapeutically to alter the growth and/or differentiation of a cell. Other aspects of the invention are described below or will be apparent to those skilled in the art in light of the present disclosure.

One aspect of the present invention relates to isolated MEKK proteins. As used herein protein, peptide, and polypeptide are meant to be synonymous. According to the present invention, an isolated protein is a protein that has been removed from its natural milieu. It will be understood that "isolated", with respect to MEKK polypeptides, is

meant to include formulations of the polypeptides which are isolated from, or otherwise substantially free of other cellular proteins ("contaminating proteins"), especially other cellular signal transduction factors, normally associated with the MEKK polypeptide. Thus, isolated MEKK protein preparations include preparations having less than 20% (by dry weight) contaminating protein, and preferably having less than 5% contaminating protein (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 5000, can be present). Functional forms of the subject MEKK polypeptides can be prepared, for the first time, as purified preparations by using a cloned gene as described herein. Alternatively, the subject MEKK polypeptides can be isolated by affinity purification using, for example, a catalytically inactive MEK. "Isolated" does not encompass either natural materials in their native state or natural materials that have been separated into components (*e.g.*, in an acrylamide gel) but not obtained either as pure (*e.g.*, lacking contaminating proteins, or chromatography reagents such as denaturing agents and polymers, *e.g.*, acrylamide or agarose) substances or solutions.

An isolated MEKK protein can, for example, be obtained from its natural source, be produced using recombinant DNA technology, or be synthesized chemically. As used herein, an isolated MEKK protein can be a full-length MEKK protein or any homologue of such a protein, such as a MEKK protein in which amino acids have been deleted (*e.g.*, a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (*e.g.*, by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycosylphosphatidyl inositol), wherein the modified protein retains a MEKK biological activity (*e.g.*, is capable of phosphorylating MAP kinase kinases, such as mitogen ERK kinases (MEKs (MKK1 and MKK2)) and/or Jun kinase kinases (JNKKs (MKK3 and MKK4)).

A homologue of a MEKK protein is a protein having an amino acid sequence that is substantially similar or homologous to a natural MEKK protein amino acid sequence that a nucleic acid sequence encoding the homologue is capable of hybridizing under stringent conditions to (*i.e.*, with) a nucleic acid sequence encoding the natural MEKK protein amino acid sequence. As used herein, stringent hybridization conditions refer to standard hybridization conditions under which nucleic acid molecules, including oligonucleotides, are used to identify similar nucleic acid molecules. Such standard conditions are disclosed, for example, in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press (1989). Exemplary stringent hybridization conditions include but are not limited to hybridization at 65°C in 4X SSC or at 42°C in 4XSSC, 50% formamide, followed by washing at 65°C in 1XSSC.

Exemplary high stringency conditions include but are not limited to hybridization at 65° C in 1XSSC or at 42°C in 1XSSC, 50% formamide followed by washing at 65°C in 0.3XsSSC. A homologue of a MEKK protein also includes a protein having an amino acid sequence that is sufficiently cross-reactive such that the homologue has the ability to elicit an immune response against at least one epitope of a naturally-occurring MEKK protein.

The minimal size of a protein homologue of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid with the complementary sequence of a nucleic acid molecule encoding the corresponding natural protein. As such, the size of the nucleic acid molecule encoding such a protein homologue is dependent on nucleic acid composition, percent homology between the nucleic acid molecule and complementary sequence, as well as upon hybridization conditions *per se* (*e.g.*, temperature, salt concentration, and formamide concentration). The minimal size of such nucleic acid molecules is typically at least about 12 to about 15 nucleotides in length if the nucleic acid molecules are GC-rich and at least about 15 to about 17 bases in length if they are AT-rich. As such, the minimal size of a nucleic acid molecule used to encode a MEKK protein homologue of the present invention is from about 12 to about 18 nucleotides in length. There is no limit, other than a practical limit, on the maximal size of such a nucleic acid molecule in that the nucleic acid molecule can include a portion of a gene, an entire gene, or multiple genes, or portions thereof. Similarly, the minimal size of a MEKK protein homologue of the present invention is from about 4 to about 6 amino acids in length, with preferred sizes depending on whether a full-length, multivalent protein (*i.e.*, fusion protein having more than one domain each of which has a function), or a functional portion of such a protein is desired.

In another embodiment, a homologue of a MEKK protein is a protein having an amino acid sequence that is at least about 60-65%, 70-75%, 80-85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% to an amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6 or a portion or fragment thereof. Alternatively, a MEKK homologue is a protein which is encoded by a nucleic acid molecule having at least 60-65%, 70-75%, 80-85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homology to a nucleic acid sequence of SEQ ID NO:3 or SEQ ID NO:5. As used herein the term "% homology" can be used interchangeably with the term "% identity".

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989) which has been incorporated into the ALIGN program (version 2.0) (available at http://vega.igh.cnrs.fr/bin/align-guess.cgi), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the

NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to MSP-18 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to MSP-18 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

MEKK protein homologues can be the result of allelic variation of a natural gene encoding a MEKK protein. A natural gene refers to the form of the gene found most often in nature. MEKK protein homologues can be produced using techniques known in the art including, but not limited to, direct modifications to a gene encoding a protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis. As will be understood, mutagenesis includes point mutations, as well as deletions and truncations of the MEKK polypeptide sequence. The ability of a MEKK protein homologue to phosphorylate MEK and/or JNKK protein can be tested using techniques known to those skilled in the art.

With respect to homologues, it will also be possible to modify the structure of the subject MEKK polypeptides for such purposes as enhancing therapeutic or prophylactic efficacy, or stability (*e.g.*, *ex vivo* shelf life and resistance to proteolytic degradation *in vivo*). Such modified polypeptides, when designed to retain at least one activity of the naturally-occurring form of the protein, are considered functional equivalents of the MEKK polypeptide described in more detail herein. Such modified peptide can be produced, for instance, by amino acid substitution, deletion, or addition.

In one aspect of the invention, the MEKK proteins and/or MEKK homologues are defined as having a MEKK "activity" or "biological activity". In one embodiment, the MEKK protein is involved in a pathway controlling the phosphorylation of a mitogen-activated protein (MAP) kinase. The mammalian MAP kinase family includes, for example, the extracellular signal-regulated protein kinases (ERK1 and ERK2), p42 or p44 MAPKs. In another preferred embodiment the MEKK protein will be involved in the pathway controlling c-Jun NH2-terminal kinases (JNKs, or SAPKs), and the so-called "p38 subgroup" kinases (p38 and Hog-1 kinases). For example, it is contemplated that the MEKK proteins of the present invention interact with, and directly phosphorylate members of the MAP kinase kinase family (MEKs or MKKs), as MEK1, MEK2, MKK1, MKK2, or the stress-activated kinases (SEKs), and the Jun kinase kinases (JNKK1, JNKK2, MKK3, MKK4), or the like. An exemplary MEKK-dependent pathway includes a pathway involving a MEKK protein and a MKK protein.

In another embodiment, a MEKK protein is capable of regulating the activity of signal transduction proteins including, but not limited to, mitogen activated ERK kinases (MEKs), mitogen activated protein kinases (MAPKs), transcription control factor (TCF), Ets-like-1 transcription factor (Elk-1), Jun ERK kinases (JNKKs), Jun kinases (JNK; which is equivalent to SAPK), stress activated MAPK proteins, Jun, activating transcription factor-2 (ATF-2) and/or Myc protein.

As used herein, the "activity" or "biological activity" of a protein can be directly correlated with the phosphorylation state of the protein and/or the ability of the protein to perform a particular function (*e.g.*, phosphorylate another protein or regulate transcription). Preferred MEK proteins regulated by a MEKK protein of the present invention include MEK-1 and/or MEK-2 (MKK1 or MKK2). Preferred MAPK proteins regulated by a MEKK protein of the present invention include p38/Hog-1 MAPK, p42 MAPK and/or p44 MAPK. Preferred stress activated MAPK proteins regulated by a MEKK protein of the present invention include Jun kinase (JNK), stress activated MAPK-α and/or stress activated MAPK-β.

In a preferred embodiment, a MEKK protein of the present invention is capable of phosphorylating a MEK or MKK, Jun kinase kinase (JNKK) and/ or stress activated ERK kinase (SEK), in particular MEK1, MEK2, MKK1, MKK2, MKK3, MKK4, JNKK1, JNKK2, SEK1 and/or SEK2 proteins. As described herein, MEK1 and MEK2 are equivalent to MKK1 and MKK2, respectively. In addition, JNKK1 and JNKK2 are equivalent to MKK3 and MKK4, which are equivalent to SEK1 and SEK2.

A preferred MEKK protein of the present invention is additionally capable of inducing Myc proteins and members of the Ets family of transcription factors, such as TCF protein, also referred to as Elk-1 protein.

Another aspect of the present invention is the recognition that a MEKK protein of the present invention is capable of regulating the apoptosis of a cell As used herein, apoptosis refers to the form of cell death that comprises: progressive contraction of cell volume with the preservation of the integrity of cytoplasmic organelles; condensation of chromatin, as viewed by light or electron microscopy; and DNA cleavage, as electrophoresis or labeling of DNA fragments using terminal deoxytransferase (TDT). Cell death occurs when the membrane integrity of the cell is lost and cell lysis occurs. Apoptosis differs from necrosis in which cells swell and eventually rupture.

A preferred MEKK protein of the present invention is capable of inducing the apoptosis of cells, such that the cells have characteristics substantially similar to cytoplasmic shrinkage and/or nuclear condensation.

A schematic representation of exemplary cell growth regulatory pathways that are MEKK dependent is shown in Figure 6.

In addition to the numerous functional characteristics of a MEKK protein, a MEKK protein of the present invention comprises numerous unique structural characteristics. For example, in one embodiment, a MEKK protein of the present invention includes at least one of two different structural domains having particular functional characteristics. Such structural domains include an NH_2-terminal regulatory domain that serves to regulate a second structural domain comprising a COOH-terminal protein kinase catalytic domain that is capable of phosphorylating an MKK protein.

According to the present invention, a MEKK protein of the present invention includes a full-length MEKK protein, as well as at least a portion of a MEKK protein capable of performing at least one of the functions defined above. Preferred portions are capable of inducing apoptosis. The phrase "at least a portion of a MEKK protein" refers to a portion of a MEKK protein encoded by a nucleic acid molecule that is capable of hybridizing, under stringent conditions, with a nucleic acid encoding a full-length MEKK protein of the present invention. Preferred portions of MEKK proteins are useful for regulating apoptosis in a cell. Suitable sizes for portions of a MEKK protein of the present invention are 65-70kD, 75-80kD, 85-90kD, 100-110kD, 120-130kD, 140-150kD, 160-170kD or 180kD or larger as determined by Tris-glycine SDS-PAGE, preferably using an 8% polyacrylamide SDS gel (SDS-PAGE) and resolved using methods standard in the art. It is noted that experimental conditions used when running gels to determine the molecular size of putative MEKK proteins and/or portions thereof will cause variations in results.

In one embodiment, a portion of a MEKK protein capable of inducing apoptosis includes about amino acids 875-1493 of murine MEKK1, set forth in SEQ ID NO:4. In another embodiment, a portion of a MEKK1 protein capable of inducing apoptosis includes about amino acids 685-1303 of human MEKK1, set forth in SEQ ID NO:6. In another embodiment, a portion of a MEKK protein capable of inducing apoptosis is substantially similar or homologous to amino acids 875-1493 of murine MEKK1, set forth in SEQ ID NO:4. In another embodiment, a portion of a MEKK protein capable of inducing apoptosis is substantially similar or homologous to amino acids 685-1303 of human MEKK1, set forth in SEQ ID NO:6.

The sequences comprising the catalytic domain of a MEKK protein are involved in phosphotransferase activity, and therefore display a relatively conserved amino acid sequence. The NH_2-terminal regulatory domain of a MEKK protein, however, can be substantially divergent. As such, the NH_2-terminal regulatory domain of a MEKK

protein provides selectivity for upstream signal transduction regulation, while the catalytic domain provides for MEKK substrate selectivity function.

In another embodiment, the subject MEKK proteins are provided as fusion proteins. It is widely appreciated that fusion proteins can also facilitate the expression of proteins, and accordingly, can be used in the expression of the MEKK polypeptides of the present invention. For example, MEKK polypeptides can be generated as glutathione-S-transferase (GST-fusion) proteins. Such GST-fusion proteins can enable easy purification of the MEKK polypeptide, as for example by the use of glutathione-derivatized matrices (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* (N.Y.: John Wiley & Sons, 1991)).

In another embodiment, a fusion gene coding for a purification leader sequence, such as a poly-(His)/enterokinase cleavage site sequence at the N-terminus of the desired portion of the recombinant protein, can allow purification of the expressed fusion protein by affinity chromatography using a Ni2+ metal resin. The purification leader sequence can then be subsequently removed by treatment with enterokinase to provide the purified protein (*e.g.*, see Hochuli *et al.* (1987) *J. Chromatography* 411:177; and Janknecht *et al. PNAS* 88:8972).

Techniques for making fusion genes are known to those skilled in the art. Essentially, the joining of various DNA fragments coding for different polypeptide sequences is performed in accordance with conventional techniques, employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992).

According to the present invention, a MEKK protein of the present invention can include MEKK proteins that have undergone post-translational modification. Such modification can include, for example, phosphorylation or among other post-translational modifications including conformational changes or post-translational deletions.

This invention further contemplates a method for generating sets of combinatorial mutants of the subject MEKK proteins as well as truncation mutants, and is especially useful for identifying potential variant sequences (*e.g.*, homologs) that are

functional in modulating signal transduction. The purpose of screening such combinatorial libraries is to generate, for example, novel MEKK homologs which can act as either agonists or antagonist of the wild-type MEKK proteins, or alternatively, which possess novel activities all together. To illustrate, MEKK homologs can be engineered by the present method to provide selective, constitutive activation of a pathway, so as mimic induction by a factor when the MEKK homolog is expressed in a cell capable of responding to the factor. Thus, combinatorially-derived homologs can be generated to have an increased potency relative to a naturally occurring form of the protein.

Likewise, MEKK homologs can be generated by the present combinatorial approach to selectively inhibit (antagonize) induction by a growth or other factor. For instance, mutagenesis can provide MEKK homologs which are able to bind other signal pathway proteins (*e.g.*, MEKs) yet prevent propagation of the signal, *e.g.*, the homologs can be dominant negative mutants. Moreover, manipulation of certain domains of MEKK by the present method can provide domains more suitable for use in fusion proteins.

In one aspect of this method, the amino acid sequences for a population of MEKK homologs or other related proteins are aligned, preferably to promote the highest homology possible. Such a population of variants can include, for example, MEKK homologs from one or more species. Amino acids which appear at each position of the aligned sequences are selected to create a degenerate set of combinatorial sequences. In a preferred embodiment, the variegated library of MEKK variants is generated by combinatorial mutagenesis at the nucleic acid level, and is encoded by a variegated gene library. For instance, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequences such that the degenerate set of potential MEKK sequences are expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of MEKK sequences therein.

There are many ways by which such libraries of potential MEKK homologs can be generated from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be carried out in an automatic DNA synthesizer, and the synthetic genes then ligated into an appropriate expression vector. The purpose of a degenerate set of genes is to provide, in one mixture, all of the sequences encoding the desired set of potential MEKK sequences. The synthesis of degenerate oligonucleotides is well known in the art (see for example, Narang, SA (1983) *Tetrahedron* 39:3; Itakura *et al.* (1981) *Recombinant DNA, Proc 3rd Cleveland Sympos. Macromolecules*, ed. AG Walton, Amsterdam: Elsevier pp273-289; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.*

11:477. Such techniques have been employed in the directed evolution of other proteins (see, for example, Scott *et al.* (1990) *Science* 249:386-390; Roberts *et al.* (1992) *PNAS* 89:2429-2433; Devlin *et al.* (1990) *Science* 249: 404-406; Cwirla *et al.* (1990) *PNAS* 87: 6378-6382; as well as U.S. Patents Nos. 5,223,409, 5,198,346, and 5,096,815).

Likewise, a library of coding sequence fragments can be provided for a MEKK clone in order to generate a variegated population of MEKK fragments for screening and subsequent selection of bioactive fragments. A variety of techniques are known in the art for generating such libraries, including chemical synthesis. In one embodiment, a library of coding sequence fragments can be generated by (i) treating a double stranded PCR fragment of a MEKK coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule; (ii) denaturing the double stranded DNA; (iii) renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products; (iv) removing single stranded portions from reformed duplexes by treatment with S1 nuclease; and (v) ligating the resulting fragment library into an expression vector. By this exemplary method, an expression library can be derived which codes for N-terminal, C-terminal and internal fragments of various sizes.

A wide range of techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a certain property. Such techniques will be generally adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of MEKK homologs. The most widely used techniques for screening large gene libraries typically comprise cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates relatively easy isolation of the vector encoding the gene whose product was detected. Each of the illustrative assays described below are amenable to high through-put analysis as necessary to screen large numbers of degenerate MEKK sequences created by combinatorial mutagenesis techniques.

In an illustrative embodiment of a screening assay, the gene library can be expressed as a fusion protein on the surface of a viral particle. For instance, in the filamentous phage system, foreign peptide sequences can be expressed on the surface of infectious phage, thereby conferring two significant benefits. First, since these phage can be applied to affinity matrices at very high concentrations, a large number of phage can be screened at one time. Second, since each infectious phage displays the combinatorial gene product on its surface, if a particular phage is recovered from an affinity matrix in low yield, the phage can be amplified by another round of infection.

The group of almost identical *E. coli* filamentous phages M13, fd, and f1 are most often used in phage display libraries, as either of the phage gIII or gVIII coat proteins can be used to generate fusion proteins without disrupting the ultimate packaging of the viral particle (Ladner *et al.* PCT publication WO 90/02909; Garrard *et al.*, PCT publication WO 92/09690; Marks *et al.* (1992) *J. Biol. Chem.* 267:16007-16010; Griffths *et al.* (1993) *EMBO J* 12:725-734; Clackson *et al.* (1991) *Nature* 352:624-628; and Barbas *et al.* (1992) *PNAS* 89:4457-4461). The resulting phage libraries with the fusion tail proteins may be panned, *e.g.*, using a fluorescent labeled MEK protein, *e.g.*, FITC-MEK, to score for MEKK homologs which retain the ability to bind to the MEK protein. Individual phage which encode a MEKK homolog which retains MEK binding can be isolated, the MEKK homolog gene recovered from the isolate, and further tested to discern between active and antagonistic mutants

In another embodiment, cells (*e.g.*, REF52 cells) can be exploited to analyze the variegated MEKK library. For instance, the library of expression vectors can be transfected into a population of REF52 cells which also inducibly overexpress a MEKK protein (*e.g.*, and which overexpression causes apoptosis). Expression of WT-MEKK is then induced. and the effect of the MEKK mutant on induction of apoptosis can be detected. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of apoptosis, and the individual clones further characterized.

The invention also provides for reduction of the MEKK proteins to generate mimetics, *e.g.*, peptide or non-peptide agents, which are able to disrupt binding of a MEKK polypeptide of the present invention with either upstream or downstream components of its signaling cascade. Thus, such mutagenic techniques as described above are also useful to map the determinants of the MEKK proteins which participate in protein-protein interactions involved in, for example, binding of the subject MEKK polypeptide to proteins which may function upstream (including both activators and repressors of its activity) or to proteins which may function downstream of the MEKK polypeptide, whether they are positively or negatively regulated by it. To illustrate, the critical residues of a subject MEKK polypeptide which are involved in molecular recognition of an upstream or downstream MEKK component can be determined and used to generate MEKK-derived peptidomimetics which competitively inhibit binding of the authentic protein with that moiety. By employing, for example, scanning mutagenesis to map the amino acid residues of each of the subject MEKK proteins which are involved in binding other cellular proteins, peptidomimetic compounds can be generated which mimic those residues of the MEKK protein which facilitate the interaction. Such mimetics may then be used to interfere with the normal function of a

MEKK protein. For instance, non-hydrolyzable peptide analogs of such residues can be generated using benzodiazepine (*e.g.*, see Freidinger *et al.* in *Peptides: Chemistry and Biology*, G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988), azepine (*e.g.*, see Huffman *et al.* in *Peptides: Chemistry and Biology*, G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988), substituted gamma lactam rings (Garvey *et al.* in *Peptides: Chemistry and Biology*, G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988), keto-methylene pseudopeptides (Ewenson *et al.* (1986) *J Med Chem* 29:295; and Ewenson *et al.* in *Peptides: Structure and Function* (Proceedings of the 9th American Peptide Symposium) Pierce Chemical Co. Rockland, IL, 1985), β-turn dipeptide cores (Nagai *et al.* (1985) *Tetrahedron Lett* 26:647; and Sato *et al.* (1986) *J Chem Soc Perkin Trans* 1:1231), and β-aminoalcohols (Gordon *et al.* (1985) *Biochem Biophys Res Commun*126:419; and Dann *et al.* (1986) *Biochem Biophys Res Commun* 134:71).

Another aspect of the present invention is an isolated nucleic acid molecule capable of hybridizing, under stringent conditions, with a MEKK protein gene encoding a MEKK protein of the present invention. In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (*i.e.*, that has been subject to human manipulation). As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. To this end, the term "isolated" as used herein with respect to nucleic acids, such as DNA or RNA, refers to molecules separated from other DNAs, or RNAs, respectively, that are present in the natural source of the macromolecule. For example, an isolated nucleic acid encoding one of the subject MEKK polypeptides preferably includes no more than 10 kilobases (kb) of nucleic acid sequence which naturally immediately flanks the MEKK gene in genomic DNA, more preferably no more than 5kb of such naturally occurring flanking sequences, and most preferably less than 1.5kb of such naturally occurring flanking sequence. In another example, the isolated MEKK nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. The term isolated as used herein will also be understood to include nucleic acid that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Moreover, an "isolated nucleic acid" is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state.

An isolated nucleic acid molecule can include DNA, RNA, or derivatives of either DNA or RNA. Accordingly, as used herein, the term "nucleic acid" includes polynucleotides such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term should also be understood to include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs, and, as applicable to the embodiment being described, single (sense or antisense) and double-stranded polynucleotides.

As used herein, the term "gene" or "recombinant gene" includes nucleic acid comprising an open reading frame encoding one of the MEKK polypeptides of the present invention, including both exon and (optionally) intron sequences. A "recombinant gene" refers to nucleic acid encoding a MEKK polypeptide (*e.g.*, a vertebrate MEKK polypeptide) and comprising MEKK-encoding exon sequences, though it may optionally include intron sequences which are either derived from a chromosomal MEKK gene or from an unrelated chromosomal gene. Exemplary recombinant genes encoding the subject MEKK polypeptides are represented in the appended Sequence Listing. The term "intron" refers to a DNA sequence present in a given MEKK gene which is not translated into protein and is generally found between exons.

An isolated nucleic acid molecule of the present invention can be obtained from its natural source either as an entire (*i.e.*, complete) gene or a portion thereof capable of forming a stable hybrid with that gene. As used herein, the phrase "at least a portion of" an entity refers to an amount of the entity that is at least sufficient to have the functional aspects of that entity. For example, at least a portion of a nucleic acid sequence, as used herein, is an amount of a nucleic acid sequence capable of forming a stable hybrid with a particular desired gene (*e.g.*, MEKK genes) under stringent hybridization conditions. An isolated nucleic acid molecule of the present invention can also be produced using recombinant DNA technology (*e.g.*, polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Isolated MEKK protein nucleic acid molecules include natural nucleic acid molecules and homologues thereof, including, but not limited to, natural allelic variants and modified nucleic acid molecules in which nucleotides have been inserted, deleted, substituted, and/or inverted in such a manner that such modifications do not substantially interfere with the nucleic acid molecule's ability to encode a MEKK protein of the present invention or to form stable hybrids under stringent conditions with natural nucleic acid molecule isolates of MEKK.

Preferred modifications to a MEKK protein nucleic acid molecule of the present invention include truncating a full-length MEKK protein nucleic acid molecule by, for example: deleting at least a portion of a MEKK protein nucleic acid molecule encoding

a regulatory domain to produce a constitutively active MEKK protein; deleting at least a portion of a MEKK protein nucleic acid molecule encoding a catalytic domain to produce an inactive MEKK protein; and modifying the MEKK protein to achieve desired inactivation and/or stimulation of the protein, for example, substituting a codon encoding a lysine residue in the catalytic domain (*i.e.*, phosphotransferase domain) with a methionine residue to inactivate the catalytic domain.

A preferred truncated MEKK nucleic acid molecule encodes a form of a MEKK protein which is capable of inducing apoptosis.

An isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one MEKK protein of the present invention, examples of such proteins being disclosed herein. Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides that comprise the nucleic acid molecule, the two phrases can be used interchangeably. As heretofore disclosed, MEKK proteins of the present invention include, but are not limited to, proteins having full-length MEKK protein coding regions, portions thereof, and other MEKK protein homologues.

As used herein, a MEKK protein gene includes all nucleic acid sequences related to a natural MEKK protein gene such as regulatory regions that control production of a MEKK protein encoded by that gene (including, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself. A nucleic acid molecule of the present invention can be an isolated natural MEKK protein nucleic acid molecule or a homologue thereof. A nucleic acid molecule of the present invention can include one or more regulatory regions, full-length or partial coding regions, or combinations thereof. The minimal size of a MEKK protein nucleic acid molecule of the present invention is the minimal size capable of forming a stable hybrid under stringent hybridization conditions with a corresponding natural gene.

A MEKK protein nucleic acid molecule homologue can be produced using a number of methods known to those skilled in the art (see, *e.g.*, Sambrook *et al.*, *ibid.*). For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis techniques and recombinant DNA techniques, such as site-directed mutagenesis, chemical treatment of a nucleic acid molecule to induce mutations, restriction enzyme cleavage of a nucleic acid fragment, ligation of nucleic acid fragments, polymerase chain reaction (PCR) amplification and/or mutagenesis of selected regions of a nucleic acid sequence, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologues can be selected from a

mixture of modified nucleic acids by screening for the function of the protein encoded by the nucleic acid (*e.g.*, the ability of a homologue to phosphorylate MEK protein or JNKK protein) and/or by hybridization with isolated MEKK protein nucleic acids under stringent conditions.

A preferred nucleic acid molecule of the present invention is capable of hybridizing under stringent conditions to a nucleic acid that encodes at least a portion of a MEKK protein, or a homologue thereof. Also preferred is a MEKK nucleic acid molecule that includes a nucleic acid sequence having at least about 50% homology, preferably 75% homology, preferably 85% homology, or even more preferably 95% homology with an MEKK nucleic acid molecule of the invention. In other embodiments nucleic acids have 50%, preferably at least about 75%, and more preferably at least about 85%, and most preferably at least about 95% homology with the corresponding region(s) of the nucleic acid sequence encoding the catalytic domain of a MEKK protein, or a homologue thereof. Also preferred is a MEKK protein nucleic acid molecule that includes a nucleic acid sequence having at least about 50%, preferably at least about 75%, more preferably at least about 85%, and even more preferably at least about 95% homology with the corresponding region(s) of the nucleic acid sequence encoding the NH_2-terminal regulatory domain of a MEKK protein, or a homologue thereof. Such nucleic acid molecules can be a full-length gene and/or a nucleic acid molecule encoding a full-length protein, a hybrid protein, a fusion protein, a multivalent protein or a truncation fragment.

Knowing a nucleic acid molecule of a MEKK protein of the present invention allows one skilled in the art to make copies of that nucleic acid molecule as well as to obtain additional portions of MEKK protein-encoding genes (*e.g.*, nucleic acid molecules that include the translation start site and/or transcription and/or translation control regions), and/or MEKK protein nucleic acid molecule homologues. Knowing a portion of an amino acid sequence of a MEKK protein of the present invention allows one skilled in the art to clone nucleic acid sequences encoding such a MEKK protein.

The present invention also includes nucleic acid molecules that are oligonucleotides capable of hybridizing, under stringent conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention that encode at least a portion of a MEKK protein, or a homologue thereof. A preferred oligonucleotide is capable of hybridizing, under stringent conditions, with a nucleic acid molecule of SEQ ID NO: 3 or SEQ ID NO:5.

Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimal size of such oligonucleotides is the size required to form a stable hybrid between a given oligonucleotide and the complementary sequence on another

nucleic acid molecule of the present invention. Minimal size characteristics of preferred oligonucleotides are at least about 10 nucleotides, preferably at least about 20 nucleotides, more preferably at least about 50 nucleotides and most preferably at least about 60 nucleotides. Larger fragments are also contemplated. The size of the oligonucleotide must also be sufficient for the use of the oligonucleotide in accordance with the present invention. Oligonucleotides of the present invention can be used in a variety of applications including, but not limited to, as probes to identify additional nucleic acid molecules, as primers to amplify or extend nucleic acid molecules or in therapeutic applications to inhibit, for example, expression of MEKK proteins by cells. Such therapeutic applications include the use of such oligonucleotides in, for example, antisense-, triplex formation-, ribozyme- and/or RNA drug-based technologies. The present invention, therefore, includes use of such oligonucleotides and methods to interfere with the production of MEKK proteins. In addition oligonucleotides encoding portions of MEKK proteins which bind to MEKK binding proteins can be used a therapeutics. In other embodiments, the peptides encoded by these nucleic acids are used.

To further illustrate, another aspect of the invention relates to the use of the isolated nucleic acid in "antisense" therapy. As used herein, "antisense" therapy refers to administration or *in situ* generation of oligonucleotide probes or their derivatives which specifically hybridize (*e.g.*, bind) under cellular conditions, with the cellular mRNA and/or genomic DNA encoding one or more of the subject MEKK proteins so as to inhibit expression of that protein, *e.g.*, by inhibiting transcription and/or translation. The binding may be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix. In general, "antisense" therapy refers to the range of techniques generally employed in the art, and includes any therapy which relies on specific binding to oligonucleotide sequences.

An antisense construct of the present invention can be delivered, for example, as an expression plasmid which, when transcribed in the cell, produces RNA which is complementary to at least a unique portion of the cellular mRNA which encodes a vertebrate MEKK protein. Alternatively, the antisense construct is an oligonucleotide probe which is generated *ex vivo* and which, when introduced into the cell causes inhibition of expression by hybridizing with the mRNA and/or genomic sequences of a vertebrate MEKK gene. Such oligonucleotide probes are preferably modified oligonucleotides which are resistant to endogenous nucleases, *e.g.*, exonucleases and/or endonucleases, and are therefore stable *in vivo*. Exemplary nucleic acid molecules for use as antisense oligonucleotides are phosphoramidate, phosphothioate and

methylphosphonate analogs of DNA (see also U.S. Patents 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy have been reviewed, for example, by Van der Krol *et al.* (1988) *Biotechniques* 6:958-976; and Stein *et al.* (1988) *Cancer Res* 48:2659-2668.

Accordingly, the modified oligomers of the invention are useful in therapeutic, diagnostic, and research contexts. In therapeutic applications, the oligomers are utilized in a manner appropriate for antisense therapy in general. For such therapy, the oligomers of the invention can be formulated for a variety of loads of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in *Remmington's Pharmaceutical Sciences*, Meade Publishing Co., Easton, PA. For systemic administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the oligomers of the invention can be formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the oligomers may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included.

Systemic administration can also be by transmucosal or transdermal means, or the compounds can be administered orally. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration may be through nasal sprays or using suppositories. For oral administration, the oligomers are formulated into conventional oral administration forms such as capsules, tablets, and tonics. For topical administration, the oligomers of the invention are formulated into ointments, salves, gels, or creams as generally known in the art.

In addition to use in therapy, the oligomers of the invention may be used as diagnostic reagents to detect the presence or absence of the target DNA or RNA sequences to which they specifically bind. Such diagnostic tests are described in further detail below.

Likewise, the antisense constructs of the present invention, by antagonizing the normal biological activity of one of the MEKK proteins, can be used in the manipulation of tissue, *e.g.*, tissue differentiation, both *in vivo* and for *ex vivo* tissue cultures.

Furthermore, the anti-sense techniques (*e.g.*, microinjection of antisense molecules, or transfection with plasmids whose transcripts are anti-sense with regard to a MEKK mRNA or gene sequence) can be used to investigate role of MEKK in disease states, as well as the normal cellular function of MEKK in healthy tissue. Such

techniques can be utilized in cell culture, but can also be used in the creation of transgenic animals. The present invention also includes a recombinant vector which includes at least one MEKK protein nucleic acid molecule of the present invention inserted into any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, for example nucleic acid sequences that are not naturally found adjacent to MEKK protein nucleic acid molecules of the present invention. The vector can be either RNA or DNA, and either prokaryotic or eukaryotic, and is typically a virus or a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulating of MEKK protein nucleic acid molecules of the present invention. One type of recombinant vector, herein referred to as a recombinant molecule and described in more detail below, can be used in the expression of nucleic acid molecules of the present invention. Preferred recombinant vectors are capable of replicating in the transformed cell.

Preferred nucleic acid molecules to insert into a recombinant vector includes a nucleic acid molecule that encodes at least a portion of a MEKK protein, or a homologue thereof. In particularly preferred embodiments portions of a MEKK nucleic acid which encodes a MEKK catalytic domain is used. In another particularly preferred embodiment, at least a portion of a nucleic acid which encodes the portion of a MEKK protein which binds to a MEKK substrate or a MEKK regulatory protein is used.

Preferred nucleic acid molecules for insertion into an expression vector include nucleic acid molecules that encode at least a portion of a MEKK protein, or a homologue thereof.

Expression vectors of the present invention may also contain fusion sequences which lead to the expression of inserted nucleic acid molecules of the present invention as fusion proteins. Inclusion of a fusion sequence as part of a MEKK nucleic acid molecule of the present invention can enhance the stability during production, storage and/or use of the protein encoded by the nucleic acid molecule. Furthermore, a fusion segment can function as a tool to simplify purification of a MEKK protein, such as to enable purification of the resultant fusion protein using affinity chromatography. A suitable fusion segment can be a domain of any size that has the desired function (*e.g.*, increased stability and/or purification tool). It is within the scope of the present invention to use one or more fusion segments. Fusion segments can be joined to amino and/or carboxyl termini of a MEKK protein. Linkages between fusion segments and MEKK proteins can be constructed to be susceptible to cleavage to enable straight-forward recovery of the MEKK proteins. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a fusion nucleic acid sequence that

encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of a MEKK protein.

Moreover, the gene constructs of the present invention can also be used as a part of a gene therapy protocol to deliver nucleic acids encoding either an agonistic or antagonistic form of one of the subject MEKK proteins. Thus, another aspect of the invention features expression vectors for *in vivo* or *in vitro* transfection and expression of a MEKK polypeptide in particular cell types so as to reconstitute the function of, constitutively activate, or alternatively, abrogate the function of a signal pathway dependent on a MEKK activity. Such therapies may useful where the naturally-occurring form of the protein is misexpressed or inappropriately activated; or to deliver a form of the protein which alters differentiation of tissue; or which inhibits neoplastic transformation.

Expression constructs of the subject MEKK polypeptide, and mutants thereof, may be administered in any biologically effective carrier, *e.g.*, any formulation or composition capable of effectively delivering the recombinant gene to cells *in vivo*. Approaches include insertion of the subject gene in viral vectors including recombinant retroviruses, adenovirus, adeno-associated virus, and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors transfect cells directly; plasmid DNA can be delivered with the help of, for example, cationic liposomes (lipofectin) or derivatized (*e.g.*, antibody conjugated), polylysine conjugates, gramacidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or $CaPO_4$ precipitation carried out *in vivo*. It will be appreciated that because transduction of appropriate target cells represents the critical first step in gene therapy, choice of the particular gene delivery system will depend on such factors as the phenotype of the intended target and the route of administration, *e.g.*, locally or systemically. Furthermore, it will be recognized that the particular gene construct provided for *in vivo* transduction of MEKK expression are also useful for *in vitro* transduction of cells, such as for use in the *ex vivo* tissue culture systems described below.

A preferred approach for *in vivo* introduction of nucleic acid into a cell is by use of a viral vector containing nucleic acid, *e.g.*, a cDNA, encoding the particular MEKK polypeptide desired. Infection of cells with a viral vector has the advantage that a large proportion of the targeted cells can receive the nucleic acid. Additionally, molecules encoded within the viral vector, *e.g.*, by a cDNA contained in the viral vector, are expressed efficiently in cells which have taken up viral vector nucleic acid.

Retrovirus vectors and adeno-associated virus vectors are generally understood to be the recombinant gene delivery system of choice for the transfer of exogenous genes *in vivo*, particularly into humans. These vectors provide efficient delivery of genes into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host. A major prerequisite for the use of retroviruses is to ensure the safety of their use, particularly with regard to the possibility of the spread of wild-type virus in the cell population. The development of specialized cell lines (termed "packaging cells") which produce only replication-defective retroviruses has increased the utility of retroviruses for gene therapy, and defective retroviruses are well characterized for use in gene transfer for gene therapy purposes (for a review see Miller, A.D. (1990) *Blood* 76:271). Thus, recombinant retrovirus can be constructed in which part of the retroviral coding sequence (*gag, pol, env*) has been replaced by nucleic acid encoding one of the subject proteins rendering the retrovirus replication defective. The replication defective retrovirus is then packaged into virions which can be used to infect a target cell through the use of a helper virus by standard techniques. Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in *Current Protocols in Molecular Biology*, Ausubel, F.M. *et al.* (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM which are well known to those skilled in the art. Examples of suitable packaging virus lines for preparing both ecotropic and amphotropic retroviral systems include ψ Crip, ψCre, ψ2 and ψAm. Retroviruses have been used to introduce a variety of genes into many different cell types, including neuronal cells, *in vitro* and/or *in vivo* (see for example Eglitis, *et al.* (1985) *Science* 230:1395-1398; Danos and Mulligan (1988) *Proc. Natl. Acad. Sci. USA* 85:6460-6464; Wilson *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:3014-3018; Armentano *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:6141-6145; Huber *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:8039-8043; Ferry *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:8377-8381; Chowdhury *et al.* (1991) *Science* 254:1802-1805; van Beusechem *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:7640-7644; Kay *et al.* (1992) *Human Gene Therapy* 3:641-647; Dai *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:10892-10895; Hwu *et al.* (1993) *J. Immunol.* 150:4104-4115; U.S. Patent No. 4,868,116; U.S. Patent No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573).

Furthermore, it has been shown that it is possible to limit the infection spectrum of retroviruses and consequently of retroviral-based vectors, by modifying the viral packaging proteins on the surface of the viral particle (see, for example PCT

publications WO93/25234 and WO94/06920). For instance, strategies for the modification of the infection spectrum of retroviral vectors include: coupling antibodies specific for cell surface antigens to the viral *env* protein (Roux *et al.* (1989) *PNAS* 86:9079-9083; Julan *et al.* (1992) *J. Gen Virol* 73:3251-3255; and Goud *et al.* (1983) *Virology* 163:251-254); or coupling cell surface receptor ligands to the viral *env* proteins (Neda *et al.* (1991) *J Biol Chem* 266:14143-14146). Coupling can be in the form of the chemical cross-linking with a protein or other variety (*e.g.*, lactose to convert the *env* protein to an asialoglycoprotein), as well as by generating fusion proteins (*e.g.*, single-chain antibody/*env* fusion proteins). This technique, while useful to limit or otherwise direct the infection to certain tissue types, can also be used to convert an ecotropic vector in to an amphotropic vector.

Moreover, use of retroviral gene delivery can be further enhanced by the use of tissue- or cell-specific transcriptional regulatory sequences which control expression of the MEKK gene of the retroviral vector.

Another viral gene delivery system useful in the present invention utilizes adenovirus-derived vectors. The genome of an adenovirus can be manipulated such that it encodes and expresses a gene product of interest but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. See for example Berkner *et al.* (1988) *Biotechniques* 6:616; Rosenfeld *et al.* (1991) *Science* 252:431-434; and Rosenfeld *et al.* (1992) *Cell* 68:143-155. Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (*e.g.*, Ad2, Ad3, Ad7 etc.) are well known to those skilled in the art. Recombinant adenoviruses can be advantageous in certain circumstances in that they can be used to infect a wide variety of cell types, including airway epithelium (Rosenfeld *et al.* (1992) cited *supra*), endothelial cells (Lemarchand *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6482-6486), hepatocytes (Herz and Gerard (1993) *Proc. Natl. Acad. Sci. USA* 90:2812-2816) and muscle cells (Quantin *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:2581-2584). Furthermore, the virus particle is relatively stable and amenable to purification and concentration, and as above, can be modified so as to affect the spectrum of infectivity. Additionally, introduced adenoviral DNA (and foreign DNA contained therein) is not integrated into the genome of a host cell but remains episomal, thereby avoiding potential problems that can occur as a result of insertional mutagenesis in situations where introduced DNA becomes integrated into the host genome (*e.g.*, retroviral DNA). Moreover, the carrying capacity of the adenoviral genome for foreign DNA is large (up to 8 kilobases) relative to other gene delivery vectors (Berkner *et al.* cited *supra*; Haj-Ahmand and Graham (1986) *J. Virol.* 57:267). Most replication-defective adenoviral vectors currently in use and therefore favored by the present invention are deleted for all or parts of the viral E1

and E3 genes but retain as much as 80% of the adenoviral genetic material (see, *e.g.*, Jones *et al.* (1979) *Cell* 16:683; Berkner *et al.*, *supra*; and Graham *et al.* in *Methods in Molecular Biology*, E.J. Murray, Ed. (Humana, Clifton, NJ, 1991) vol. 7. pp. 109-127). Expression of the inserted MEKK gene can be under control of, for example, the E1A promoter, the major late promoter (MLP) and associated leader sequences, the E3 promoter, or exogenously added promoter sequences.

Yet another viral vector system useful for delivery of one of the subject MEKK genes is the adeno-associated virus (AMINO ACIDSV). Adeno-associated virus is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle. (For a review see Muzyczka *et al. Curr. Topics in Micro. and Immunol.* (1992) 158:97-129). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (see for example Flotte *et al.* (1992) *Am. J. Respir. Cell. Mol. Biol.* 7:349-356; Samulski *et al.* (1989) *J. Virol.* 63:3822-3828; and McLaughlin *et al.* (1989) *J. Virol.* 62:1963-1973). Vectors containing as little as 300 base pairs of AMINO ACIDSV can be packaged and can integrate. Space for exogenous DNA is limited to about 4.5 kb. An AMINO ACIDSV vector such as that described in Tratschin *et al.* (1985) *Mol. Cell. Biol.* 5:3251-3260 can be used to introduce DNA into cells. A variety of nucleic acids have been introduced into different cell types using AMINO ACIDSV vectors (see for example Hermonat *et al.* (1984) *Proc. Natl. Acad. Sci. USA* 81:6466-6470; Tratschin *et al.* (1985) *Mol. Cell. Biol.* 4:2072-2081; Wondisford *et al.* (1988) *Mol. Endocrinol.* 2:32-39; Tratschin *et al.* (1984) *J. Virol.* 51:611-619; and Flotte *et al.* (1993) *J. Biol. Chem.* 268:3781-3790).

In addition to viral transfer methods, such as those illustrated above, non-viral methods can also be employed to cause expression of a subject MEKK polypeptide in the tissue of an animal. Most nonviral methods of gene transfer rely on normal mechanisms used by mammalian cells for the uptake and intracellular transport of macromolecules. In preferred embodiments, non-viral gene delivery systems of the present invention rely on endocytic pathways for the uptake of the subject MEKK polypeptide gene by the targeted cell. Exemplary gene delivery systems of this type include liposomal derived systems, poly-lysine conjugates, and artificial viral envelopes.

In clinical settings, the gene delivery systems for the therapeutic MEKK gene can be introduced into a patient by any of a number of methods, each of which is familiar in the art. For instance, a pharmaceutical preparation of the gene delivery system can be introduced systemically, *e.g.*, by intravenous injection, and specific transduction of the protein in the target cells occurs predominantly from specificity of transfection provided by the gene delivery vehicle, cell-type or tissue-type expression

due to the transcriptional regulatory sequences controlling expression of the receptor gene, or a combination thereof. In other embodiments, initial delivery of the recombinant gene is more limited with introduction into the animal being quite localized. For example, the gene delivery vehicle can be introduced by catheter (see U.S. Patent 5,328,470) or by stereotactic injection (*e.g.*, Chen *et al.* (1994) *PNAS* 91: 3054-3057). A MEKK gene, such as any one of the clones represented in the appended Sequence Listing, can be delivered in a gene therapy construct by electroporation using techniques described, for example, by Dev *et al.* ((1994) *Cancer Treat Rev* 20:105-115).

The pharmaceutical preparation of the gene therapy construct can consist essentially of the gene delivery system in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery system can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can comprise one or more cells which produce the gene delivery system.

Still another aspect of the present invention pertains to recombinant cells, *e.g.*, cells which are transformed with at least one of any nucleic acid molecule of the present invention. A preferred recombinant cell is a cell transformed with at least one nucleic acid molecule that encodes at least a portion of a MEKK protein, or a homologue thereof.

Suitable host cells for transforming a cell can include any cell capable of producing MEKK proteins of the present invention after being transformed with at least one nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule. Suitable host cells of the present invention can include bacterial, fungal (including yeast), insect, animal and plant cells. Preferred host cells include bacterial, yeast, insect and mammalian cells, with mammalian cells being particularly preferred.

A recombinant cell is preferably produced by transforming a host cell with one or more recombinant molecules, each comprising one or more nucleic acid molecules of the present invention operatively linked to an expression vector containing one or more transcription control sequences. The phrase operatively linked refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. Expression vectors can be either prokaryotic or eukaryotic, and are typically viruses or plasmids. Expression vectors of the present invention include any vectors that function (*i.e.*, direct gene

expression) in recombinant cells of the present invention, including in bacterial, fungal, insect, animal, and/or plant cells. As such, nucleic acid molecules of the present invention can be operatively linked to expression vectors containing regulatory sequences such as promoters, operators, repressors, enhancers, termination sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of nucleic acid molecules of the present invention. As used herein, a transcription control sequence includes a sequence which is capable of controlling the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in bacterial, yeast, and mammalian cells, such as, but not limited to, *tac*, *lac*, *trp*, *trc*, oxy-pro, omp/lpp, rrnB, bacteriophage lambda (λ) (such as λp_L and λp_R and fusions that include such promoters), bacteriophage T7, T7*lac*, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha mating factor, baculovirus, vaccinia virus, herpesvirus, poxvirus, adenovirus, simian virus 40, retrovirus actin, retroviral long terminal repeat, Rous sarcoma virus, heat shock, phosphate and nitrate transcription control sequences, as well as other sequences capable of controlling gene expression in prokaryotic or eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (*e.g.*, promoters inducible by interferons or interleukins). Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with a DNA sequence encoding a MEKK protein.

It may be appreciated by one skilled in the art that use of recombinant DNA technologies can improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control

signals (*e.g.*, promoters, operators, enhancers), substitutions or modifications of translational control signals (*e.g.*, ribosome binding sites, Shine-Dalgarno sequences), modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, deletion of sequences that destabilize transcripts, and use of control signals that temporally separate recombinant cell growth from recombinant protein production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing the resultant protein.

As used herein, amplifying the copy number of a nucleic acid sequence in a cell can be accomplished either by increasing the copy number of the nucleic acid sequence in the cell's genome or by introducing additional copies of the nucleic acid sequence into the cell by transformation. Copy number amplification is conducted in a manner such that greater amounts of enzyme are produced, leading to enhanced conversion of substrate to product. For example, recombinant molecules containing nucleic acids of the present invention can be transformed into cells to enhance enzyme synthesis. Transformation can be accomplished using any process by which nucleic acid sequences are inserted into a cell. Prior to transformation, the nucleic acid sequence on the recombinant molecule can be manipulated to encode an enzyme having a higher specific activity.

In accordance with the present invention, recombinant cells can be used to produce a MEKK protein of the present invention by culturing such cells under conditions effective to produce such a protein, and recovering the protein. Effective conditions to produce a protein include, but are not limited to, appropriate media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An appropriate, or effective, medium refers to any medium in which a cell of the present invention, when cultured, is capable of producing a MEKK protein. Such a medium is typically an aqueous medium comprising assimilable carbohydrate, nitrogen and phosphate sources, as well as appropriate salts, minerals, metals and other nutrients, such as vitamins. The medium may comprise complex nutrients or may be a defined minimal medium.

A preferred cell to culture is a recombinant cell that is capable of expressing the MEKK protein, the recombinant cell being produced by transforming a host cell with one or more nucleic acid molecules of the present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue,

organ or a multicellular organism. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (*i.e.*, recombinant) cell in such a manner that their ability to be expressed is retained.

With respect to methods for producing the subject MEKK polypeptide, a host cell transfected with a nucleic acid vector directing expression of a nucleotide sequence encoding the subject polypeptides can be cultured under appropriate conditions to allow expression of the peptide to occur. The cells may be harvested, lysed and the protein isolated. A cell culture includes host cells, media and other byproducts. Suitable media for cell culture are well known in the art. The recombinant MEKK polypeptide can be isolated from cell culture medium, host cells, or both using techniques known in the art for purifying proteins including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, and immunoaffinity purification with antibodies specific for such peptide. In a preferred embodiment, the recombinant MEKK polypeptide is a fusion protein containing a domain which facilitates its purification, such as GST fusion protein or poly(His) fusion protein.

Cells of the present invention can be cultured in conventional fermentation bioreactors, which include, but are not limited to, batch, fed-batch, cell recycle, and continuous fermentors. Culturing can also be conducted in shake flasks, test tubes, microtiter dishes, and petri plates. Culturing is carried out at a temperature, pH and oxygen content appropriate for the recombinant cell. Such culturing conditions are well within the expertise of one of ordinary skill in the art.

Depending on the vector and host system used for production, resultant MEKK proteins may either remain within the recombinant cell or be secreted into the fermentation medium. The phrase "recovering the protein" refers simply to collecting the whole fermentation medium containing the protein and need not imply additional steps of separation or purification. MEKK proteins of the present invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, chromatofocusing and differential solubilization.

Alternatively, a MEKK protein of the present invention can be produced by isolating the MEKK protein from cells or tissues recovered from an animal that normally express the MEKK protein. For example, a cell type, such as T cells, can be isolated from the thymus of an animal. MEKK protein can then be isolated from the isolated primary T cells using standard techniques described herein.

The availability of purified and recombinant MEKK polypeptides as described in the present invention facilitates the development of assays which can be used to screen for drugs, including MEKK homologs, which are either agonists or antagonists of the normal cellular function of the subject MEKK polypeptides, or of their role in the pathogenesis of cellular differentiation and/or proliferation, and disorders related thereto. In one embodiment, the assay evaluates the ability of a compound to modulate binding between a MEKK polypeptide and a molecule that interacts either upstream or downstream of the MEKK polypeptide in the a cellular signaling pathway. A variety of assay formats will suffice and, in light of the present inventions, will be comprehended by a skilled artisan.

In many drug screening programs which test libraries of compounds and natural extracts, high throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins, are often preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound. Moreover, the effects of cellular toxicity and/or bioavailability of the test compound can be generally ignored in the *in vitro* system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity with upstream or downstream elements.

Accordingly, in an exemplary screening assay of the present invention, the compound of interest is contacted with proteins which may function upstream (including both activators and repressors of its activity such as, Ras, Rac, Cdc 42 or Rho or other Ras superfamily members) or to proteins or nucleic acids which may function downstream of the MEKK polypeptide, whether they are positively or negatively regulated by it. For convenience, such polypeptides of a signal transduction pathway which interact directly with MEKK will be referred to below as MEKK-binding proteins (MEKK-bp). These proteins include the downstream targets of MEKKs, namely, members of the MAP kinase kinase family (MEKs or MKKs), as MEK1, MEK2, MKK1, MKK2, the stress-activated kinases (SEKs), also known as the Jun kinase kinases (JNKKs), MEKK3 and MEKK4 or the like. Other downstream targets of the MEKK family can include proteins from the mammalian MAP kinase family which includes, for example, the extracellular signal-regulated protein kinases (ERKs), c-Jun NH_2-terminal kinases (JNKs, or SAPKs), and the so-called "p38 subgroup" kinases (p38 kinases).

To the mixture of the compound and the MEKK-bp is then added a composition containing a MEKK polypeptide. Detection and quantification of complexes including MEKK and the MEKK-bp provide a means for determining a compound's efficacy at inhibiting (or potentiating) complex formation between MEKK and the MEKK-binding protein. The efficacy of the compound can be assessed by generating dose response curves from data obtained using various concentrations of the test compound. Moreover, a control assay can also be performed to provide a baseline for comparison. In the control assay, isolated and purified MEKK polypeptide is added to a composition containing the MEKK-binding protein, and the formation of a complex is quantitated in the absence of the test compound.

Complex formation between the MEKK polypeptide and a MEKK-binding protein may be detected by a variety of techniques. Modulation of the formation of complexes can be quantitated using, for example, detectably labeled proteins such as radiolabeled, fluorescently labeled, or enzymatically labeled MEKK polypeptides, by immunoassay, or by chromatographic detection.

Typically, it will be desirable to immobilize either MEKK or its binding protein to facilitate separation of complexes from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of the two proteins, in the presence and absence of a candidate agent, can be accomplished in any vessel suitable for containing the reactants. Examples include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows the protein to be bound to a matrix. For example, glutathione-S-transferase/MEKK (GST/MEKK) fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the MEKK-bp, *e.g.*, an ^{35}S-labeled, and the test compound, and the mixture incubated under conditions conducive to complex formation, *e.g.*, at physiological conditions for salt and pH, though slightly more stringent conditions may be desired. Following incubation, the beads are washed to remove any unbound label, and the matrix immobilized and radiolabel determined directly (*e.g.*, beads placed in scintilant), or in the supernatant after the complexes are subsequently dissociated. Alternatively, the complexes can be dissociated from the matrix, separated by SDS-PAGE, and the level of MEKK-binding protein found in the bead fraction quantitated from the gel using standard electrophoretic techniques such as described in the appended examples.

Other techniques for immobilizing proteins on matrices are also available for use in the subject assay. For instance, either MEKK or its cognate binding protein can be immobilized utilizing conjugation of biotin and streptavidin. For instance, biotinylated

MEKK molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with MEKK but which do not interfere with binding of upstream or downstream elements can be derivatized to the wells of the plate, and MEKK trapped in the wells by antibody conjugation. As above, preparations of a MEKK-binding protein and a test compound are incubated in the MEKK-presenting wells of the plate, and the amount of complex trapped in the well can be quantitated. Exemplary methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the MEKK binding protein, or which are reactive with the MEKK protein and compete with the binding protein; as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the binding protein, either intrinsic or extrinsic activity. In the instance of the latter, the enzyme can be chemically conjugated or provided as a fusion protein with the MEKK-bp. To illustrate, the MEKK-bp can be chemically cross-linked or genetically fused with horseradish peroxidase, and the amount of polypeptide trapped in the complex can be assessed with a chromogenic substrate of the enzyme, *e.g.*, 3,3'-diamino-benzadine terahydrochloride or 4-chloro-1-napthol. Likewise, a fusion protein comprising the polypeptide and glutathione-S-transferase can be provided, and complex formation quantitated by detecting the GST activity using 1-chloro-2,4-dinitrobenzene (Habig et al (1974) J Biol Chem 249:7130).

For processes which rely on immunodetection for quantitating one of the proteins trapped in the complex, antibodies against the protein, such as anti-MEKK antibodies, can be used. Alternatively, the protein to be detected in the complex can be "epitope tagged" in the form of a fusion protein which includes, in addition to the MEKK sequence, a second polypeptide for which antibodies are readily available (*e.g.*, from commercial sources). For instance, the GST fusion proteins described above can also be used for quantification of binding using antibodies against the GST moiety. Other useful epitope tags include myc-epitopes (*e.g.*, see Ellison *et al.* (1991) J Biol Chem 266:21150-21157) which includes a 10-residue sequence from c-myc, as well as the pFLAG system (International Biotechnologies, Inc.) or the pEZZ-protein A system (Pharamacia, NJ).

In addition to cell-free assays, such as described above, the readily available source of vertebrate MEKK proteins provided by the present invention also facilitates the generation of cell-based assays for identifying small molecule agonists/antagonists and the like. Cells which are sensitive to MEKK-mediated signal transduction events

can be caused to overexpress a recombinant MEKK protein in the presence and absence of a test agent of interest, with the assay scoring for modulation in MEKK-dependent responses by the target cell mediated by the test agent. As with the cell-free assays, agents which produce a statistically significant change in MEKK-dependent signal transduction (either inhibition or potentiation) can be identified.

In another embodiment of a drug screening, a two hybrid assay can be generated with a MEKK and MEKK-binding protein. This assay permits the detection of protein-protein interactions in yeast such that drug dependent inhibition or potentiation of the interaction can be scored. As an illustrative example, GAL4 protein is a potent activator of transcription in yeast grown on galactose. The ability of GAL4 to activate transcription depends on the presence of an N-terminal sequence capable of binding to a specific DNA sequence (UASG) and a C-teminal domain containing a transcriptional activator. A sequence encoding a MEKK protein, "A", may be fused to that encoding the DNA binding domain of the GAL4 protein. A second hybrid protein may be created by fusing sequence encoding the GAL4 transactivation domain to sequence encoding a MEKK-bp, "B". If protein "A" and protein "B" interact, that interaction serves to bring together the two domains of GAL4 necessary to activate transcription of a UASG-containing gene. In addition to co-expressing plasmids encoding both hybrid proteins, yeast strains appropriate for the detection of protein-protein interactions would contain, for example, a GAL1-lacZ fusion gene to permit detection of transcription from a UASG sequence. Other examples of two-hybrid assays or interaction trap assays are known in the art.

In an illustrative embodiment, a portion of MEKK4 providing a Rac/Cdc42 binding site is provided in one fusion protein, along with a second fusion protein including a Rac/Cdc42 polypeptide. This embodiment of the subject assay permits the screening of compounds which inhibit or potentiate the binding of MEKK4 and Cdc42.

Phosphorylation assays may also be used. MEKK binding proteins can be tested for their ability to phosphorylate substrates in addition, compounds that inhibit or activate MEKK regulated pathways and phenotypic responses can be tested.

Furthermore, each of the assay systems set out above can be generated in a "differential" format. That is, the assay format can provide information regarding specificity as well as potency. For instance, side-by-side comparison of a test compound's effect on different MEKKs can provide information on selectivity, and permit the identification of compounds which selectively modulate the bioactivity of only a subset of the MEKK family.

The present invention also includes a method to identify compounds capable of regulating signals initiated from a receptor on the surface of a cell, such signal regulation involving in some respect, MEKK protein. Such a method comprises the steps of: (a) contacting a cell containing a MEKK protein with a putative regulatory compound; (b) contacting the cell with a ligand capable of binding to a receptor on the surface of the cell; and (c) assessing the ability of the putative regulatory compound to regulate cellular signals by determining activation of a member of a MEKK-dependent pathway of the present invention. A preferred method to perform step (c) comprises measuring the phosphorylation of a member of a MEKK-dependent pathway. Such measurements can be performed using immunoassays having antibodies specific for phosphotyrosines, phosphoserines and/or phosphothreonines. Another preferred method to perform step (c) comprises measuring the ability of the MEKK protein to phosphorylate a substrate molecule comprising a protein including MKK1, MKK2, MKK3, or MKK4, Raf-1, Ras-GAP and neurofibromin using methods described herein. Preferred substrates include MEK1, MEK2, JNKK1 and JNKK2. Yet another preferred method to perform step (c) comprises determining the ability
of MEKK protein to bind to Ras, rac or Cdc 42 protein. In particular, determining the ability of MEKK protein to bind to GST-Ras^{V12}(GTPγS) or GST-Rac^{v14}(GTPγS).

Putative compounds as referred to herein include, for example, compounds that are products of rational drug design, natural products and compounds having partially defined signal transduction regulatory properties. A putative compound can be a protein-based compound, a carbohydrate-based compound, a lipid-based compound, a nucleic acid-based compound, a natural organic compound, a synthetically derived organic compound, an anti-idiotypic antibody and/or catalytic antibody, or fragments thereof. A putative regulatory compound can be obtained, for example, from libraries of natural or synthetic compounds, in particular from chemical or combinatorial libraries (*i.e.*, libraries of compounds that differ in sequence or size but that have the same building blocks; see for example, U.S. Patent Nos. 5,010,175 and 5,266,684 of Rutter and Santi) or by rational drug design.

Preferred MEKK protein for use with the method includes recombinant MEKK protein. More preferred MEKK protein includes at least a portion of a MEKK protein having a kinase domain or apoptotic domain of MEKK.

Another aspect of the present invention includes a kit to identify compounds capable of regulating signals initiated from a receptor on the surface of a cell, such signals involving in some respect, MEKK protein. Such kits include: (a) at least one cell containing MEKK protein; (b) a ligand capable of binding to a receptor on the surface of the cell; and (c) a means for assessing the ability of a putative regulatory compound to

alter phosphorylation of the MEKK protein. Such a means for detecting phosphorylation include methods and reagents known to those of skill in the art, for example, phosphorylation can be detected using antibodies specific for phosphorylated amino acid residues, such as tyrosine, serine and threonine. Using such a kit, one is capable of determining, with a fair degree of specificity, the location along a signal transduction pathway of particular pathway constituents, as well as the identity of the constituents involved in such pathway, at or near the site of regulation.

In another embodiment, a kit of the present invention can include: (a) MEKK protein; (b) MEKK substrate, such as MEK; and (c) a means for assessing the ability of a putative inhibitory compound to inhibit phosphorylation of the MEKK substrate by the MEKK protein.

In yet another embodiment, a mammalian MEKK gene can be used to rescue a yeast cell having a defective ste11 (or byr2) gene, such as a temperature sensitive mutant ste11 mutant (cf., Francois *et al.* (1991) *J Biol Chem* 266:6174-80; and Jenness *et al.* (1983) *Cell* 35:521-9). For example, a humanized yeast can be generated by amplifying the coding sequence of the human MEKK clone, and subcloning this sequence into a vector which contains a yeast promoter and termination sequences flanking the MEKK coding sequences. This plasmid can then be used to transform an ste11^{TS} mutant. To assay growth rates, cultures of the transformed cells can be grown at an permissive temperature for the TS mutant. Turbidity measurements, for example, can be used to easily determine the growth rate. At the non-permissive temperature, pheromone responsiveness of the yeast cells becomes dependent upon expression of the human MEKK protein. Accordingly, the humanized yeast cells can be utilized to identify compounds which inhibit the action of the human MEKK protein. It is also deemed to be within the scope of this invention that the humanized yeast cells of the present assay can be generated so as to comprise other human cell-cycle proteins. For example, human MEK and human MAPK can also be expressed in the yeast cell in place of ste7 and Fus3/Kss1. In this manner, the reagent cells of the present assay can be generated to more closely approximate the natural interactions which the mammalian MEKK protein might experience.

Furthermore, certain formats of the subject assays can be used to identify drugs which inhibit proliferation of yeast cells or other lower eukaryotes, but which have a substantially reduced effect on mammalian cells, thereby improving therapeutic index of the drug as an anti-mycotic agent. For instance, in one embodiment, the identification of such compounds is made possible by the use of differential screening assays which detect and compare drug-mediated disruption of binding between two or more different types of MEKK/MEKK-bp complexes, or which differentially inhibit the kinase activity

of, for example, ste11 relative to a mammalian MEKK. Differential screening assays can be used to exploit the difference in drug-mediated disruption of human MEKK complexes and yeast ste11/byr2 complexes in order to identify agents which display a statistically significant increase in specificity for disrupting the yeast complexes (or kinase activity) relative to the human complexes. Thus, lead compounds which act specifically to inhibit proliferation of pathogens, such as fungus involved in mycotic infections, can be developed. By way of illustration, the present assays can be used to screen for agents which may ultimately be useful for inhibiting at least one fungus implicated in such mycosis as candidiasis, aspergillosis, mucomycosis, blastomycosis, geotrichosis, cryptococcosis, chromoblastomycosis, coccidioidomycosis, conidiosporosis, histoplasmosis, maduromycosis, rhinosporidosis, nocaidiosis, para-actinomycosis, penicilliosis, monoliasis, or sporotrichosis. For example, if the mycotic infection to which treatment is desired is candidiasis, the present assay can comprise comparing the relative effectiveness of a test compound on mediating disruption of a human MEKK with its effectiveness towards disrupting the equivalent ste11/byr2 kinase from genes cloned from yeast selected from the group consisting of *Candida albicans*, *Candida stellatoidea*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida pseudotropicalis*, *Candida quillermondii*, or *Candida rugosa*. Likewise, the present assay can be used to identify anti-fungal agents which may have therapeutic value in the treatment of aspergillosis by making use of genes cloned from yeast such as *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus nidulans*, or *Aspergillus terreus*. Where the mycotic infection is mucormycosis, the complexes can be derived from yeast such as *Rhizopus arrhizus*, *Rhizopus oryzae*, *Absidia corymbifera*, *Absidia ramosa*, or *Mucor pusillus*. Sources of other ste11/byr2 homologs for comparison with a human MEKK includes the pathogen *Pneumocystis carinii*.

Another aspect of the present invention relates to the treatment of an animal having a medical disorder that is subject to regulation or cure by manipulating a signal transduction pathway in a cell involved in the disorder. Such medical disorders include disorders which result from abnormal cellular growth or abnormal production of secreted cellular products. In particular, such medical disorders include, but are not limited to, cancer, autoimmune disease, inflammatory responses, allergic responses and neuronal disorders, such as Parkinson's disease and Alzheimer's disease. Preferred cancers subject to treatment using a method of the present invention include, but are not limited to, small cell carcinomas, non-small cell lung carcinomas with overexpressed EGF receptors, breast cancers with overexpressed EGF or Neu receptors, tumors having overexpressed growth factor receptors of established autocrine loops and tumors having overexpressed growth factor receptors of established paracrine loops. According to the

present invention, the term treatment can refer to the regulation of the progression of a medical disorder or the complete removal of a medical disorder (*e.g.*, cure). Treatment of a medical disorder can comprise regulating the signal transduction activity of a cell in such a manner that a cell involved in the medical disorder no longer responds to extracellular stimuli (*e.g.*, growth factors or cytokines), or the killing of a cell involved in the medical disorder through cellular apoptosis.

The present invention relates to a method of inducing and/or maintaining a differentiated state, enhancing survival, and/or promoting (or alternatively inhibiting) proliferation of a cell responsive to a growth factor, morphogen or other environmental cue which effects the cell through at least one signal transduction pathway which includes a MEKK protein. In general, the method comprises contacting the cells with an amount of an agent which significantly (statistical) modulates MEKK-dependent signaling by the factor. For instance, it is contemplated by the invention that, in light of the present finding of an apparently broad involvement of members of the MEKK protein family in signal pathways implicated in the formation of ordered spatial arrangements of differentiated tissues in vertebrates, the subject method could be used to generate and/or maintain an array of different vertebrate tissue both *in vitro* and *in vivo*. A "MEKK therapeutic," whether inductive or anti-inductive with respect to signaling by a MEKK-dependent pathway, can be, as appropriate, any of the preparations described above, including isolated polypeptides, gene therapy constructs, antisense molecules, peptidomimetics or agents identified in the drug assays provided herein.

There are a wide variety of pathological cell proliferative conditions for which MEKK therapeutics of the present invention can be used in treatment. For instance, such agents can provide therapeutic benefits where the general strategy being the inhibition of an anomalous cell proliferation. Diseases that might benefit from this methodology include, but are not limited to various cancers and leukemias, psoriasis, bone diseases, fibroproliferative disorders such as involving connective tissues, atherosclerosis and other smooth muscle proliferative disorders, as well as chronic inflammation.

In addition to proliferative disorders, the present invention contemplates the use of MEKK therapeutics for the treatment of differentiative disorders which result from, for example, de-differentiation of tissue which may (optionally) be accompanied by abortive reentry into mitosis, *e.g.*, apoptosis. Such degenerative disorders include chronic neurodegenerative diseases of the nervous system, including Alzheimer's disease, Parkinson's disease, Huntington's chorea, amylotrophic lateral sclerosis and the like, as well as spinocerebellar degenerations. Other differentiative disorders include, for example, disorders associated with connective tissue, such as may occur due to de-

differentiation of chondrocytes or osteocytes, as well as vascular disorders which involve de-differentiation of endothelial tissue and smooth muscle cells, gastric ulcers characterized by degenerative changes in glandular cells, and renal conditions marked by failure to differentiate, *e.g.*, Wilm's tumors.

It will also be apparent that, by transient use of modulators of MEKK pathways, *in vivo* reformation of tissue can be accomplished, *e.g.*, in the development and maintenance of organs. By controlling the proliferative and differentiative potential for different cells, the subject MEKK therapeutics can be used to reform injured tissue, or to improve grafting and morphology of transplanted tissue. For instance, MEKK agonists and antagonists can be employed in a differential manner to regulate different stages of organ repair after physical, chemical or pathological insult. For example, such regimens can be utilized in repair of cartilage, increasing bone density, liver repair subsequent to a partial hepatectomy, or to promote regeneration of lung tissue in the treatment of emphysema.

Another aspect of the present invention concerns transgenic animals which are comprised of cells (of that animal) which contain a transgene of the present invention and which preferably (though optionally) express an exogenous MEKK protein in one or more cells in the animal. A MEKK transgene can encode the wild-type form of the protein, or can encode homologs thereof, including both agonists and antagonists, as well as antisense constructs. In preferred embodiments, the expression of the transgene is restricted to specific subsets of cells, tissues or developmental stages utilizing, for example, cis-acting sequences that control expression in the desired pattern. In the present invention, such mosaic expression of a MEKK protein can be essential for many forms of lineage analysis and can additionally provide a means to assess the effects of, for example, lack of MEKK expression which might grossly alter development in small patches of tissue within an otherwise normal embryo. Toward this and, tissue-specific regulatory sequences and conditional regulatory sequences can be used to control expression of the transgene in certain spatial patterns. Moreover, temporal patterns of expression can be provided by, for example, conditional recombination systems or prokaryotic transcriptional regulatory sequences.

Genetic techniques which allow for the expression of transgenes can be regulated via site-specific genetic manipulation *in vivo* are known to those skilled in the art. For instance, genetic systems are available which allow for the regulated expression of a recombinase that catalyzes the genetic recombination a target sequence. As used herein, the phrase "target sequence" refers to a nucleotide sequence that is genetically recombined by a recombinase. The target sequence is flanked by recombinase recognition sequences and is generally either excised or inverted in cells expressing

recombinase activity. Recombinase catalyzed recombination events can be designed such that recombination of the target sequence results in either the activation or repression of expression of one of the subject MEKK proteins. For example, excision of a target sequence which interferes with the expression of a recombinant MEKK gene, such as one which encodes an antagonistic homolog or an antisense transcript, can be designed to activate expression of that gene. This interference with expression of the protein can result from a variety of mechanisms, such as spatial separation of the MEKK gene from the promoter element or an internal stop codon. Moreover, the transgene can be made wherein the coding sequence of the gene is flanked by recombinase recognition sequences and is initially transfected into cells in a 3' to 5' orientation with respect to the promoter element. In such an instance, inversion of the target sequence will reorient the subject gene by placing the 5' end of the coding sequence in an orientation with respect to the promoter element which allow for promoter driven transcriptional activation.

In an illustrative embodiment, either the *cre/loxP* recombinase system of bacteriophage P1 (Lakso *et al.* (1992) *PNAS* 89:6232-6236; Orban *et al.* (1992) *PNAS* 89:6861-6865) or the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355; PCT publication WO 92/15694) can be used to generate *in vivo* site-specific genetic recombination systems. Cre recombinase catalyzes the site-specific recombination of an intervening target sequence located between *loxP* sequences. *loxP* sequences are 34 base pair nucleotide repeat sequences to which the Cre recombinase binds and are required for Cre recombinase mediated genetic recombination. The orientation of *loxP* sequences determines whether the intervening target sequence is excised or inverted when Cre recombinase is present (Abremski *et al.* (1984) *J. Biol. Chem.* 259:1509-1514); catalyzing the excision of the target sequence when the *loxP* sequences are oriented as direct repeats and catalyzes inversion of the target sequence when *loxP* sequences are oriented as inverted repeats.

Accordingly, genetic recombination of the target sequence is dependent on expression of the Cre recombinase. Expression of the recombinase can be regulated by promoter elements which are subject to regulatory control, *e.g.*, tissue-specific, developmental stage-specific, inducible or repressible by externally added agents. This regulated control will result in genetic recombination of the target sequence only in cells where recombinase expression is mediated by the promoter element. Thus, the activation expression of a recombinant MEKK protein can be regulated via control of recombinase expression.

Use of the *cre/loxP* recombinase system to regulate expression of a recombinant MEKK protein requires the construction of a transgenic animal containing transgenes encoding both the Cre recombinase and the subject protein. Animals containing both

the Cre recombinase and a recombinant MEKK gene can be provided through the construction of "double" transgenic animals. A convenient method for providing such animals is to mate two transgenic animals each containing a transgene, *e.g.*, a MEKK gene and recombinase gene.

One advantage derived from initially constructing transgenic animals containing a MEKK transgene in a recombinase-mediated expressible format derives from the likelihood that the subject protein, whether agonistic or antagonistic, can be deleterious upon expression in the transgenic animal. In such an instance, a founder population, in which the subject transgene is silent in all tissues, can be propagated and maintained. Individuals of this founder population can be crossed with animals expressing the recombinase in, for example, one or more tissues and/or a desired temporal pattern. Thus, the creation of a founder population in which, for example, an antagonistic MEKK transgene is silent will allow the study of progeny from that founder in which disruption of MEKK mediated induction in a particular tissue or at certain developmental stages would result in, for example, a lethal phenotype.

Similar conditional transgenes can be provided using prokaryotic promoter sequences which require prokaryotic proteins to be simultaneous expressed in order to facilitate expression of the MEKK transgene. Exemplary promoters and the corresponding trans-activating prokaryotic proteins are given in U.S. Patent No. 4,833,080.

Moreover, expression of the conditional transgenes can be induced by gene therapy-like methods wherein a gene encoding the trans-activating protein, *e.g.*, a recombinase or a prokaryotic protein, is delivered to the tissue and caused to be expressed, such as in a cell-type specific manner. By this method, a MEKK transgene could remain silent into adulthood until "turned on" by the introduction of the trans-activator.

In an exemplary embodiment, the "transgenic non-human animals" of the invention are produced by introducing transgenes into the germline of the non-human animal. Embryonic target cells at various developmental stages can be used to introduce transgenes. Different methods are used depending on the stage of development of the embryonic target cell. The zygote is the best target for micro-injection. In the mouse, the male pronucleus reaches the size of approximately 20 micrometers in diameter which allows reproducible injection of 1-2pl of DNA solution. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the injected DNA will be incorporated into the host gene before the first cleavage (Brinster *et al.* (1985) *PNAS* 82:4438-4442). As a consequence, all cells of the transgenic non-human animal will carry the incorporated transgene. This will in general also be reflected in the efficient

transmission of the transgene to offspring of the founder since 50% of the germ cells will harbor the transgene. Microinjection of zygotes is the preferred method for incorporating transgenes in practicing the invention.

Retroviral infection can also be used to introduce MEKK transgenes into a non-human animal. The developing non-human embryo can be cultured *in vitro* to the blastocyst stage. During this time, the blastomeres can be targets for retroviral infection (Jaenich, R. (1976) *PNAS* 73:1260-1264). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona pellucida (*Manipulating the Mouse Embryo*, Hogan eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1986). The viral vector system used to introduce the transgene is typically a replication-defective retrovirus carrying the transgene (Jahner *et al.* (1985) *PNAS* 82:6927-6931; Van der Putten *et al.* (1985) *PNAS* 82:6148-6152). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Van der Putten, *supra*; Stewart *et al.* (1987) *EMBO J.* 6:383-388). Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoele (Jahner *et al.* (1982) *Nature* 298:623-628). Most of the founders will be mosaic for the transgene since incorporation occurs only in a subset of the cells which formed the transgenic non-human animal. Further, the founder may contain various retroviral insertions of the transgene at different positions in the genome which generally will segregate in the offspring. In addition, it is also possible to introduce transgenes into the germ line by intrauterine retroviral infection of the midgestation embryo (Jahner *et al.* (1982) *supra*).

A third type of target cell for transgene introduction is the embryonic stem cell (ES). ES cells are obtained from pre-implantation embryos cultured *in vitro* and fused with embryos (Evans *et al.* (1981) *Nature* 292:154-156; Bradley *et al.* (1984) *Nature* 309:255-258; Gossler *et al.* (1986) *PNAS* 83: 9065-9069; and Robertson *et al.* (1986) *Nature* 322:445-448). Transgenes can be efficiently introduced into the ES cells by DNA transfection or by retrovirus-mediated transduction. Such transformed ES cells can thereafter be combined with blastocysts from a non-human animal. The ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal. For review see Jaenisch, R. (1988) *Science* 240:1468-1474.

Methods of making MEKK knock-out or disruption transgenic animals are also generally known. See, for example, *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Recombinase dependent knockouts can also be generated, *e.g.*, by homologous recombination to insert recombinase target sequences flanking portions of an endogenous MEKK gene, such

that tissue specific and/or temporal control of inactivation of a MEKK allele can be controlled as above.

According to the present invention, an isolated, or biologically pure, peptide, is a peptide that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the protein has been purified. An isolated compound of the present invention can be obtained from a natural source or produced using recombinant DNA technology or chemical synthesis. As used herein, an isolated peptide can be a full-length protein or any homolog of such a protein in which amino acids have been deleted (*e.g.*, a truncated version of the protein), inserted, inverted, substituted and/or derivatized (*e.g.*, by glycosylation, phosphorylation, acetylation, myristylation, prenylation, palmitoylation, and/or amidation) such that the peptide is capable of regulating the binding of Ras superfamily protein to MEKK protein.

In accordance with the present invention, a "mimetope" refers to any compound that is able to mimic the ability of an isolated compound of the present invention. A mimetope can be a peptide that has been modified to decrease its susceptibility to degradation but that still retain regulatory activity. Other examples of mimetopes include, but are not limited to, protein-based compounds, carbohydrate-based compounds, lipid-based compounds, nucleic acid-based compounds, natural organic compounds, synthetically derived organic compounds, anti-idiotypic antibodies and/or catalytic antibodies, or fragments thereof. A mimetope can be obtained by, for example, screening libraries of natural and synthetic compounds as disclosed herein that are capable of inhibiting the binding of Ras superfamily protein to MEKK. A mimetope can also be obtained by, for example, rational drug design. In a rational drug design procedure, the three-dimensional structure of a compound of the present invention can be analyzed by, for example, nuclear magnetic resonance (NMR) or x-ray crystallography. The three-dimensional structure can then be used to predict structures of potential mimetopes by, for example, computer modeling. The predicted mimetope structures can then be produced by, for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source (*e.g.*, plants, animals, bacteria and fungi).

The therapeutic methods of the present invention may also comprise injecting an area of a subject's body with an effective amount of a naked plasmid DNA compound (such as is taught, for example in Wolff *et al.*, 1990, *Science 247*, 1465-1468). A naked plasmid DNA compound comprises a nucleic acid molecule encoding a MEKK protein of the present invention, operatively linked to a naked plasmid DNA vector capable of being taken up by and expressed in a recipient cell located in the body area. A preferred

naked plasmid DNA compound of the present invention comprises a nucleic acid molecule encoding a truncated MEKK protein having deregulated kinase activity. Preferred naked plasmid DNA vectors of the present invention include those known in the art. When administered to a subject, a naked plasmid DNA compound of the present invention transforms cells within the subject and directs the production of at least a portion of a MEKK protein or RNA nucleic acid molecule that is capable of regulating the apoptosis of the cell.

A naked plasmid DNA compound of the present invention is capable of treating a subject suffering from a medical disorder including cancer, autoimmune disease, inflammatory responses, allergic responses and neuronal disorders, such as Parkinson's disease and Alzheimer's disease. For example, a naked plasmid DNA compound can be administered as an anti-tumor therapy by injecting an effective amount of the plasmid directly into a tumor so that the plasmid is taken up and expressed by a tumor cell, thereby killing the tumor cell. As used herein, an effective amount of a naked plasmid DNA to administer to a subject comprises an amount needed to regulate or cure a medical disorder the naked plasmid DNA is intended to treat, such mode of administration, number of doses and frequency of dose capable of being decided upon, in any given situation, by one of skill in the art without resorting to undue experimentation.

An isolated compound of the present invention can be used to formulate a therapeutic composition. In one embodiment, a therapeutic composition of the present invention includes at least one isolated peptide of the present invention. A therapeutic composition for use with a treatment method of the present invention can further comprise suitable excipients. A therapeutic compound for use with a treatment method of the present invention can be formulated in an excipient that the subject to be treated can tolerate. Examples of such excipients include water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful excipients include suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while examples of preservatives include thimerosal, m- or o-cresol, formalin and benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be taken up in a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise

dextrose, human serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

In another embodiment, a therapeutic compound for use with a treatment method of the present invention can also comprise a carrier. Carriers are typically compounds that increase the half-life of a therapeutic compound in the treated animal. Suitable carriers include, but are not limited to, liposomes, micelles, cells, polymeric controlled release formulations, biodegradable implants, bacteria, viruses, oils, esters, and glycols. Preferred carriers include liposomes and micelles.

A therapeutic compound for use with a treatment method of the present invention can be administered to any subject having a medical disorder as herein described. Acceptable protocols by which to administer therapeutic compounds of the present invention in an effective manner can vary according to individual dose size, number of doses, frequency of dose administration, and mode of administration. Determination of such protocols can be accomplished by those skilled in the art without resorting to undue experimentation. An effective dose refers to a dose capable of treating a subject for a medical disorder as described herein. Effective doses can vary depending upon, for example, the therapeutic compound used, the medical disorder being treated, and the size and type of the recipient animal. Effective doses to treat a subject include doses administered over time that are capable of regulating the activity, including growth, of cells involved in a medical disorder. For example, a first dose of a naked plasmid DNA compound of the present invention can comprise an amount that causes a tumor to decrease in size by about 10% over 7 days when administered to a subject having a tumor. A second dose can comprise at least the same the same therapeutic compound than the first dose.

Another aspect of the present invention includes a method for prescribing treatment for subjects having a medical disorder as described herein. A preferred method for prescribing treatment comprises: (a) measuring the MEKK protein activity in a cell involved in the medical disorder to determine if the cell is susceptible to treatment using a method of the present invention; and (b) prescribing treatment comprising regulating the activity of a MEKK-dependent pathway relative to the activity of a Raf-dependent pathway in the cell to induce the apoptosis of the cell. The step of measuring MEKK protein activity can comprise: (1) removing a sample of cells from a subject; (2) stimulating the cells with a TNFα; and (3) detecting the state of phosphorylation of MKK3, MKK4 or JNKK protein using an immunoassay using antibodies specific for phosphothreonine and/or phosphoserine.

The present invention also includes antibodies capable of selectively binding to a MEKK protein of the present invention. Such an antibody is herein referred to as an anti-MEKK antibody. Polyclonal populations of anti-MEKK antibodies can be contained in a MEKK antiserum. MEKK antiserum can refer to affinity purified polyclonal antibodies, ammonium sulfate cut antiserum or whole antiserum. As used herein, the term "selectively binds to" refers to the ability of such an antibody to preferentially bind to MEKK proteins. Binding can be measured using a variety of methods known to those skilled in the art including immunoblot assays, immunoprecipitation assays, enzyme immunoassays (*e.g.*, ELISA), radioimmunoassays, immunofluorescent antibody assays and immunoelectron microscopy; see, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989.

Antibodies of the present invention can be either polyclonal or monoclonal antibodies and can be prepared using techniques standard in the art. Antibodies of the present invention include functional equivalents such as antibody fragments and genetically-engineered antibodies, including single chain antibodies, that are capable of selectively binding to at least one of the epitopes of the protein used to obtain the antibodies. Preferably, antibodies are raised in response to proteins that are encoded, at least in part, by a MEKK nucleic acid molecule. More preferably antibodies are raised in response to at least a portion of a MEKK protein, and even more preferably antibodies are raised in response to either the amino terminus or the carboxyl terminus of a MEKK protein. Preferably, an antibody of the present invention has a single site binding affinity of from about 10^3M^{-1} to about $10^{12}M^{-1}$ for a MEKK protein of the present invention.

A preferred method to produce antibodies of the present invention includes administering to an animal an effective amount of a MEKK protein to produce the antibody and recovering the antibodies. Antibodies of the present invention have a variety of potential uses that are within the scope of the present invention. For example, such antibodies can be used to identify unique MEKK proteins and recover MEKK proteins.

Another aspect of the present invention comprises a therapeutic compound capable of regulating the activity of a MEKK-dependent pathway in a cell identified by a process, comprising: (a) contacting a cell with a putative regulatory molecule; and (b) determining the ability of the putative regulatory compound to regulate the activity of a MEKK-dependent pathway in the cell by measuring the activation of at least one member of said MEKK-dependent pathway. Preferred methods to measure the activation of a member of a MEKK-dependent pathway include measuring the

transcription regulation activity of c-Myc protein, measuring the phosphorylation of a protein selected from the group consisting of MEKK, JNKK, JNK, Jun, ATF-2, Myc, and combinations thereof.

The foregoing description of the invention has been presented for purposes of illustration and description. Further, the description is not intended to limit the invention to the form disclosed herein. Consequently, variations and modifications commensurate with the above teachings, and the skill or knowledge in the relevant art are within the scope of the present invention. The preferred embodiment described herein above is further intended to explain the best mode known of practicing the invention and to enable others skilled in the art to utilize the invention in various embodiments and with various modifications required by their particular applications or uses of the invention. It is intended that the appended claims be construed to include alternate embodiments to the extent permitted by the prior art.

EXAMPLES

The following examples describe the isolation and cloning of a human and murine MEKK1 nucleic acid molecule as and characterize the encoded MEKK1 proteins as well as apoptotic fragments of MEKK1 proteins. Additional exemplification of MEKK1 proteins and activities can be found in U.S. Patent Nos. 5,405,941, 5,854,043, and 5,753,446, in published PCT international application Nos. WO 94/24159 and WO95/28421, as well as in the following publications:

Russell *et al.* (1995) *Journal of Biological Chemistry* 270(20):11757-11760
Lin *et al.* (1995) *Science* 268:286-290
Johnson *et al.* (1996) *Journal of Biological Chemistry* 271(6):3229-3237
Gardner *et al.* (1994) *Molecular Biology of the Cell* 5:193-201
Blumer *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:4925-4929
Johnson (1995) U.S. Patent No. 5,405,941
Lange-Carter *et al.* (1993) *Science* 260:315-319
Lange-Carter *et al.* (1994) *Science* 265:1458-1461
Minden *et al.* (1994) *Science* 266:1719-1723.

Example 1. Isolation and Cloning of human and murine MEKK1 proteins.

MEKK1 Nucleotide Sequences

A partial murine MEKK1 nucleotide sequences, and encoded protein, was cloned and has previously been described in U.S. Patent No. 5,405,941, which is incorporated

herein by this reference. The partial murine MEKK1 nucleotide sequence is shown in SEQ ID NO: 1. The predicted amino acid sequence is shown in SEQ ID NO2. Additional cloning based on the sequence of the partial murine MEKK1 shown in SEQ ID NO:1 resulted in the nucleotide sequence of a full-length murine MEKK1 DNA which is set forth in Figure 2 and as SEQ ID NO:3. The predicted amino acid sequence of full-length murine MEKK1 is set forth as SEQ ID NO:4.

Cloning of human MEKK1

cDNA Preparation — Total mRNA was extracted and isolated from T47D cells using 1 x 10^7 cells per purification in the QuickPrep Micro mRNA Purification Kit (Pharmacia). First strand cDNA was produced using 33 microliters of the purified mRNA per reaction in the Ready-to-Go T-Primed First-Strand Kit (Pharmacia).

PCR Amplification — The sense strand primer 5'-GAACACCATCCAGAAGTTTG-3' (SEQ ID NO:13), which was designed from the mouse MEKK1 (mMEKK1) cDNA sequence, was used in conjunction with the antisense primer 5'-CACTTTGTAGACAGGGTCAGC-3' (SEQ ID NO:14) in a polymerase chain reaction (PCR) using the first strand cDNA described above as a template (RT-PCR) to amplify the region from bases 1211-1950. *Taq* DNA Polymerase (Boehringer Mannheim) was used in a RT-PCR of 30 cycles (1 min. 94°C; 1 min. 50°C; 3 min., 72°C), followed by a 10 min. incubation at 72°C. A band of approximately 800 bp was isolated by purification from a 1% agarose gel and ligated overnight at 14°C into pGEM-T coli by heat shock at 42°C, and plated on Luria Broth (LB) plates containing ampicillin and X-gal. Colonies were screened by blue/white color selection, grown up in 5 ml of LB containing ampicillin, and the plasmid DNA was isolated using the Wizard Mini-pre Kit (Promega). Isolates were then screened for insert size by digesting with PstI and AatII (Promega), and running on a 1% agarose gel. Appropriately sized inserts were sequenced from both ends using T7 and SP6 vector primers. The resulting sequence was aligned to the known mMEKK1 sequence, and determined to be hMEKK1 by homology. In order to amplify the region from bases 2263-3743, the sense primer 5'-TGGGTCGCCTCTGTCTTATAGACAG-3' (SEQ ID NO:15) was used in conjunction with the antisense primer 5'-CACATCCTGTGCTTGGTAAC-3' (SEQ ID NO:16) in a RT-PCR of 30 cycles (1 min. 94°C; 1 min., 50°C; 2 min., 72°C), followed by a 10 min. incubation at 72°C. A band of approximately 1.5 kb was isolated by purification from a 1% agarose gel, ligated, cloned, and sequenced as stated above. In order to amplify the 3' region of hMEKK1 from bases 3304-4493, the sense primer 5'-AGGACAAGTGCAGGTTAGATG-3' (SEQ ID NO:17) was used in a RT-PCR of 30 cycles (1 min., 94°C; 1 min., 50°C; 2 min., 72°C), followed by a 10 min. incubation at 72°C. A band of approximately 1.3 kb was isolated by purification from a 1% agarose gel, ligated, cloned, and sequenced as stated above. Sequence was also confirmed for

this clone using the internal sequencing primer 5'-GCTGTCCATATCTACAGTGCT-3' (SEQ ID NO:18). In order to amplify the region from bases 580-1310, the sense primer 5'-CGGCCTGGAAGCACGAGTGGT-3' (SEQ ID NO:19) was used in conjunction with the antisense primer 5'-TTCATCCTTGATGCTGTTTTC-3' (SEQ ID NO:20) in a RT-PCR of 30 cycles (1 min., 94°C; 1 min., 50°C; 2 min., 72°C), followed by a 10 min. incubation at 72°C. A band of approximately 700 bp was isolated by purification from a 1% agarose gel, ligated, cloned, and sequenced as stated above. The overlapping sequence data was compiled into a single contig using Sequencer 2.0 (Gene Codes), and aligned to the mMEKK1 sequence.

A BLAST search using the amino acid sequences of murine MEKK1 and human MEKK1 as described in this example reveals nucleotide and amino acid sequences having substantial homology to those set forth in SEQ ID NOs:3-6 (*e.g.*, sequences having Accession No. 423499, Accession No. 2507203 and Accession No. U23470).

Example 2: Apoptotic Fragments of MEKK1

This example demonstrates that MEK kinase 1 (MEKK1), a 196 kDa protein kinase, functions to integrate proteases and signal transduction pathways involved in the regulation of apoptosis. Cleavage of mouse MEKK1 at Asp^{874} generates a 91 kDa kinase fragment and a 113 kDa NH_2-terminal fragment. The kinase fragment of MEKK1 induces apoptosis. Cleavage of MEKK1 and apoptosis are inhibited by p35 and CrmA, viral inhibitors of the ICE/FLICE proteases that commit cells to apoptosis. Mutation of the MEKK1 sequence ^{871}DTVD^{874} (SEQ ID NO: 7), a cleavage site for CCP32-like proteases, to alanines inhibited proteolysis of MEKK1 and apoptosis induced by overexpression of MEKK1. Inhibition of MEKK1 proteolysis inhibited apoptosis but did not block MEKK1 stimulation of c-Jun kinase activity, indicating that c-Jun kinase activation was not sufficient for apoptosis. During the apoptotic response to UV irradiation, cisplatin, etoposide and mitomycin C, MEKK1 undergoes a phosphorylation-dependent activation followed by its proteolysis. These results show that MEKK1 activation and cleavage occurs in response to genotoxic agents and the activated kinase fragment functions to commit cells to apoptosis.

Publications referred to in these examples are abbreviated using the first author's name and the year of publication. A list of the full citation of each publication referred to in this example is provided at the end of the example.

Apoptosis or programmed cell death is a physiological process important in differentiation and tissue modeling (Williams and Smith, 1993; Steller, 1995). Apoptosis can be triggered by many different stimuli including growth factor deprivation (Xia *et al.*, 1995; Park *et al.*, 1996), exposure of specific cell types to cytokines such as TNFα and Fas ligand (Vandenabeele *et al.*, 1995; Kägi *et al.*, 1994; Lowin *et al.*, 1994), virus

infection (Esolen *et al.*, 1995; Hinshaw *et al.*, 1994; Terai *et al.*, 1991; Tyler *et al.*, 1995), and DNA damaging agents including irradiation and chemicals that induce DNA adducts (Canman and Kastan, 1996). Proteases of the ICE/FLICE family are activated during the apoptotic response that cleave specific protein substrates resulting in an irreversible commitment to cell death. Several ICE/FLICE substrates have been identified including poly (ADP-ribose) polymerase (Lazebnik *et al.*, 1994), U1 small nuclear ribonucleoprotein (Casciola-Rosen *et al.*, 1994), lamin (Lazebnik *et al.*, 1995), D4-GDI (Na *et al.*, 1996), fodrin (Cryns *et al.*, 1996), protein kinase Cδ (Emoto *et al.*, 1995), sterol regulatory element binding protein (Wang *et al.*, 1996), retinoblastoma protein (An and Dou, 1996), DNA-dependent protein kinase (Casciola-Rosen *et al.*, 1995), and the proteases themselves (Orth *et al.*, 1996).

Two ICE-like protease activities appear necessary for the apoptotic response, each with a specific substrate selectivity. ICE-like proteases such as Ced-3 have a specificity for proteins encoding the four amino acid sequence YVAD (SEQ ID NO: 10) (Howard *et al.*, 1991) while CPP32-like proteases have a preference for the sequence DEVD (SEQ ID NO: 11) (Nicholson *et al.*, 1995). Both groups of proteases cleave at the terminal aspartic acid residue of the recognition sequence. Several viruses encode proteins that are specific inhibitors of the ICE/FLICE proteases. Most notably CrmA is a poxvirus protein that inhibits ICE-like proteases, and p35 is a baculovirus protein that has broad inhibitory activity to ICE/FLICE-like proteases (Fraser and Evan, 1996; Clem *et al.*, 1996). Expression of CrmA and p35 inhibit the apoptotic response to many different stimuli demonstrating the requirement of ICE/FLICE proteases during programmed cell death (Beidler *et al.*, 1996; Los *et al.*, 1995).

In addition to ICE/FLICE proteases, it is becoming increasingly clear that signal transduction pathways involving specific protein kinases are involved in mediating apoptosis. Specifically, the c-Jun kinases (JNK) and p38 kinases have been proposed to mediate apoptosis (Verheij *et al.*, 1996; Xia *et al.*, 1995). However, a number of reports have challenged the notion that activation of JNKs and/or p38 is sufficient to induce apoptosis (Lassignal Johnson *et al.*, 1996; Tsubata *et al.*, 1993; Liu *et al.*, 1996a; Juo *et al.*, 1997; Liu *et al.*, 1996b; Park *et al.*, 1996). It appears thus that other signal pathways are required for apoptosis. However, the integration and balance of the JNK and p38 pathways probably does contribute to the commitment to apoptosis (Xia *et al.*, 1995; Gardner and Johnson, 1996).

Several protein serine-threonine kinases referred to as MEK kinases (MEKKs) have been cloned that are members of sequential protein kinase pathways regulating MAP kinases including the c-Jun kinases and ERKs [(Lange-Carter *et al.*, 1993; Lange-Carter and Johnson, 1994; Xu *et al.*, 1996; Blank *et al.*, 1996)]. In our hands, MEKKs

do not significantly activate p38 kinases. Of the four MEKK members we have characterized, MEKK1 has been found to have the unique property of being a strong stimulator of apoptosis (Lassignal Johnson *et al.*, 1996; Xia *et al.*, 1995). The other MEKKs, even though they all activate c-Jun kinases and ERKs to different levels, do not induce apoptosis, suggesting MEKK1 has unique substrates that mediate the death response. The kinase domain of MEKK1 is only 50% conserved relative to the kinase domains of MEKK 2, 3 and 4, consistent with MEKK1 having unique substrate recognition properties and catalytic activity involved in mediating the apoptotic response. MEKK1 is a 196 kDa protein that encodes a protease cleavage sequence for CPP32-like proteases. None of the other MEKKs or known kinases that regulate MAPK pathways have a consensus ICE/FLICE cleavage site. We demonstrate in this example that MEKK1 is a substrate for proteases inhibited by the p35 baculovirus protein. When the kinase domain is released from the holo-MEKK1 protein it functions as a physiological activator of apoptosis. UV irradiation and DNA damaging chemicals activate MEKK1 kinase activity and induce its proteolytic cleavage indicating that MEKK1 contributes to apoptosis in response to environmental stresses.

Materials and Methods for this Example:

Cells

Human embryonal kidney 293 cells (HEK293) stably expressing the EBNA-1 protein from Epstein-Barr virus (Invitrogen) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 U/ml penicillin/streptomycin and containing 10% bovine calf serum (BCS). The cells were transfected using lipofectamine (Gibco).

Plasmids

The full length cDNA encoding mouse MEKK1 was modified by addition of the HA-tag sequence (MGYPYDVDYAS) (SEQ ID NO: 12) at its NH_2-terminus and inserted into the expression plasmid pCEP4 (Invitrogen), resulting in plasmid MEKK1.cp4. The MEKK1 sequences DTVD (amino acids 871-874) and DEVE (amino acids 857-860) in MEKK1.cp4 were substituted with alanines using a PCR strategy. The resulting plasmids were named DTVD_A.cp4 and DEVE_A.cp4. The cDNAs for CrmA (Pickup *et al.*, 1986), p35 (Cartier *et al.*, 1994), JNK1-APF (Dérijard *et al.*, 1994) and JNK2-APF (Kallunki *et al.*, 1994) were subcloned in pCEP4 in which the hygromycin resistance gene had been removed, resulting in plasmids CrmA.cp_, p35.cp_, JNK1_APF.cp_ and JNK2_APF.cp_. Plasmid pCDNA_3.cp4 is the result of the ligation of pCEP4 and pCDNA-3.

In vitro kinase assays

Lysis buffer (70 mM β-glycerophosphate, 1 mM EGTA, 100 μM Na_3VO_4, 1 mM DTT, 2 mM $MgCl_2$, 0.5% Triton-X100, 20 μg/ml aprotinin) was added to cells 15-24 hours after transfection. Cellular debris was removed by centrifugation at 8,000xg for 5 min. Protein concentration was normalized by Bradford assay using BSA as standard.

c-Jun Kinase

c-Jun kinase (JNK) activity was measured using a solid phase kinase assay in which glutathione S-transferase-c-$Jun_{(1-79)}$ (GST-Jun) bound to glutathione-Sepharose 4B beads was used to affinity-purify JNK from cell lysates as described (Gardner and Johnson, 1996; Hibi *et al.*, 1993). Alternatively, JNK1 or JNK2 were immunoprecipitated with isoform specific antibodies (Santa Cruz Biotechnology) and GST-Jun used as substrate in an *in vitro* kinase assay (Hibi *et al.*, 1993). Quantitation of the phosphorylation of GST-Jun was performed with a PhosphorImager.

ERK

ERK2 was immunoprecipitated as described above for the JNK isoforms using the ERK2 (C-14) antibody (Santa Cruz Biotechnology). The beads were washed twice with 1 ml lysis buffer and twice with 1 ml lysis buffer without Triton-X100. Thirty-five μl of the last wash was left in the tube and mixed with 20 μl of kinase 2X mix (50 mM β-glycerophosphate, 100 μM Na_3VO_4, 20 mM $MgCl_2$, 200 μM ATP, 1 μCi/μl γ^{32}P-ATP, 400 μM EGF receptor peptide 662-681, 100 μg/μl IP-20, 2 mM EGTA), incubated 20 min at 20°C and spotted on P81 Whatman paper. The samples were washed thrice for 5 min each in 75 mM phosphoric acid and once for 2 min in acetone, air-dried, and their radioactivity determined in a β counter.

SEK1 K→M phosphorylation

MEKK1 was immunoprecipitated from cell lysates (200-500 μg) with the antibodies raised against specific sequences of MEKK1 or the 12CA5 antibody that recognizes the HA-tag sequence. The immunoprecipitates were used in an *in vitro* kinase assay with recombinant kinase inactive SEK1 (SEK1 K→M) as previously described (Blank *et al.*, 1996).

MEKK1 staining and terminal-deoxy-transferase (TdT)-mediated incorporation of fluorescent dUTP

Cells were grown on glass coverslips and transfected using lipofectamine. Two days after transfection, the medium was removed and the cells were fixed in 2% paraformaldehyde, 3% sucrose in phosphate buffered saline (PBS) for 10 min at room temperature. Following three washes with PBS, the cells were permeabilized for 10 min with 2% Triton-X100 in PBS. After three PBS washes, the cells were blocked with filtered cultured medium for 15 min. The coverslips were then incubated 1 hour in TdT reaction mix (200 mM potassium cacodylate, 25 mM Tris·HCl, pH 6.6, 250 μg/ml BSA, 5 mM $CoCl_2$, 0.25 U/μl TdT [Boehringer], 10 μM biotin-dUTP [Boehringer]) at 37 °C in a humidified atmosphere. After three washes in PBS, the coverslips were incubated for 1 hour at room temperature with a 1/500 dilution in filtered culture medium of an affinity purified rabbit antisera directed at the peptide DRPPSRELLKHPVFR of mouse MEKK1 (amino acids 1476-1490) (Lange-Carter *et al.*, 1993). The coverslips were then washed 6x over a 30 min period with PBS and incubated 1 hour at room temperature with a 1/1000 dilution in filtered culture medium of a donkey anti-rabbit, Cy^3-conjugated, antibody (Jackson Immunological) mixed with 5 μg/ml streptavidin conjugated with FITC (Jackson Immunological). The coverslips were washed 6x with PBS and incubated overnight in PBS before being mounted in 20 mg/ml o-phenyldiamine-diHCl (Sigma) in 0.1 M Tris pH 8.5, 90% glycerol. Images were taken using a Leica DM*RXA* microscope and analyzed with the SlideBook v2.0 software (Intelligent Imaging Innovations, Denver). The subcellular localization of endogenous MEKK1 observed with the anti-COOH-terminal MEKK1 antibody was identical to that observed with a second antibody recognizing the NH_2-terminal portion of the MEKK1 protein.

Immunoblots

200-400 μg cell lysate protein was subjected to SDS-9% PAGE and transferred to nitrocellulose membranes. Blots were performed exactly as described (Widmann *et al.*, 1995). To detect HA-tagged proteins, the mouse monoclonal antibody 12CA5 (Babco) was used as the primary antibody, followed by a rabbit anti-mouse antibody (Cappel). HRP-conjugated protein A at a 1/5000 dilution (Zymed) and ^{125}I-protein A at a 1/500 dilution (Dupont NEN) were then used for enhanced chemiluminescence (ECL) detection and for quantification using the PhosphorImager. To detect MEKK1, 3 different polyclonal antisera were used as primary antibodies, followed by ECL detection using HRP-protein A (see above). These sera were generated by injecting rabbits with GST proteins fused with different portions of the MEKK1 protein.

PP-2A treatment.

MEKK1 was immunoprecipitated from cell lysates (200-500 μg) using the 96-001 (NH_2) antisera, washed twice with 1 ml extraction buffer (BE) [1% Triton-X100; 10 mM Tris pH 7.4; 50 mM Nalco; 50 mM AF; 5 mM EDTA], twice with 1 ml CT (50 mM Tris pH 7.0; 0.1 mM $call_2$) and once with 1 ml CT containing 60 mM β-mercaptoethanol, 1 mM $MgCl_2$. 35 μl of the last wash was left in the tube and 0.5 U of PP-2A (Upstate Biotechnology) was added for 30-45 min. The phosphatase reaction was terminated by adding 1 μl of 200 mM Na_3VO_4. For *in vitro* kinase assay, the immunoprecipitates were washed three more times with 1 ml PAN (10 mM PIPES; 100 mM NaCl; 20 μg/ml aprotinin) before being mixed with the SEK1 K(M substrate and γ^{32}P-ATP.

Results

Expression of the 196 kDa MEKK1 protein by gene transfection induces apoptosis.

Expression of the 37 kDa kinase domain of MEKK1 (ΔMEKK1) induces cell death by apoptosis (Lassignal Johnson *et al.*, 1996; Xia *et al.*, 1995). To assess whether the full length protein had the same effect, HEK293 cells were transfected with a plasmid encoding the mouse MEKK1 and stained 2 days later for MEKK1 expression using an antibody directed at the COOH-terminus of the protein. To monitor cell death, DNA fragmentation, a feature often associated with apoptosis, was measured by terminal-deoxy-transferase-mediated incorporation of fluorescent dUTP. A large proportion of HEK293 cells expressing MEKK1 had fragmented DNA. The MEKK1 expressing cells characteristically rounded up and began to lift off the coverslips. MEKK1 also induced chromatin condensation and the nuclei in these cells often dissociated from the surrounding cytoplasm. Quantitation of cells exhibiting DNA fragmentation and cells expressing MEKK1 revealed that about 30% of MEKK1-expressing cells were apoptotic after 48 hr. This is an underestimate because the apoptotic cells eventually detach from the coverslips and often loose their nucleus. Thus, expression of the 196 kDa MEKK1 protein by gene transfection induced cell death characteristic of apoptosis similar to that observed for the 37 kDa kinase domain. The kinase activity of MEKK1 is required for the induction of cell death (Lassignal Johnson *et al.*, 1996).

MEKK1-induced DNA fragmentation is inhibited by p35 and CrmA.

Inhibition of cysteine proteases of the ICE family by the baculovirus p35 protein or by the poxvirus CrmA protein has been shown to protect cells from apoptosis in response to diverse stimuli (Beidler *et al.*, 1996). Cotransfection of HEK293 cells with MEKK1 and p35 inhibited the DNA fragmentation seen with expression of MEKK1 alone. Cotransfection of MEKK1 with CrmA also inhibited DNA fragmentation, but to a lesser extent. While only about 5% of the cells cotransfected with MEKK1 and p35 showed some DNA fragmentation, this proportion increased to about 15% in MEKK1- and CrmA-cotransfected cells. (Control cells transfected with MEKK1 alone showed about 30% DNA fragmentation). A small area of fragmented DNA was typically seen in the nucleus of these cells. Thus CrmA appears to be less efficient in protecting cells from MEKK1-induced apoptosis. Interestingly, co-expression of inhibitory mutants of the c-Jun kinases (JNK1-APF and JNK2-APF) with MEKK1 had no or only modest effects on MEKK1-mediated apoptosis. JNK1-APF expression had no effect and JNK2-APF had only a 30% diminution of apoptotic cells induced by MEKK1 expression.

CrmA and p35 inhibit cleavage of the 196 kDa MEKK1 protein and generation of an activated kinase fragment.

When MEKK1 was expressed by transfection of HEK293 cells, two additional smaller immunoreactive polypeptides besides the full length protein (named A, ~140kD, and B, ~110kD), were detected by Western blot using an antibody directed to the HA tag of MEKK1 (12CA5 antibody). The 12CA5 antibody recognizes the first 11 amino acids at the NH_2-terminus of the tagged MEKK1 protein, indicating that smaller fragments A and B must be the result of proteolysis of the full length MEKK1 protein and cannot have arisen from other potential translation sites. When an antibody directed at the COOH-terminus of MEKK1 was used (95-012 antibody), additional smaller immunoreactive fragments were also detected. Based on their apparent molecular weight, two of these fragments, named C, ~90kD, and D,~70kD, are the corresponding moieties of the cleavage products B and A, respectively. It is also important to note that the proteolytic activity can generate fragment D from fragment C. The observation that MEKK1 can be proteolyzed to very specific fragments prompted a determination of whether p35 or CrmA could inhibit the generation of fragments A, B, C and D. p35 almost totally, and CrmA partially, inhibited the appearance of fragments B and C. Quantitation of the fragments in 6 independent experiments revealed that CrmA and p35, while leaving the amount of fragment A unchanged, diminished the amount of fragment B by 50% and 90%, respectively. This indicates that these protease inhibitors prevented the formation of fragments B and C, but had no effect on the proteolytic

activity that cleaves MEKK1 into fragment A. Since the cleavage of MEKK1 into fragment A was unaffected by CrmA and p35, it was surprising to find that the amount of fragment D, the corresponding moiety of fragment A, was reduced in the presence of the inhibitors. However, because the amounts of fragments A and B formed in MEKK1-transfected cells are not significantly different from one another, the observation that there is far less fragment D than fragment C suggests that fragment D may be unstable and rapidly degraded. Moreover, since fragment D can be derived from fragment C, blocking the generation of fragment C will result in less fragment D. Neither JNK1-APF nor JNK2-APF expression influenced the generation of MEKK1 fragments, suggesting that blunting the activation of the JNK1/JNK2 pathways had little effect on the proteolysis of the MEKK1 protein.

To determine whether the cleavage of MEKK1 into fragments A, B, C and D had any effect on the kinase activity of MEKK1, lysates from cells transfected with HA-tagged MEKK1 alone or in combination with CrmA or p35 were used for immunoprecipitation with the 12CA5 HA antibody or with an antibody specific for the COOH-terminal moiety of MEKK1 (antibody 95-012). The immunoprecipitates were then incubated with a MEKK1 substrate (SEK1 K(M) and γ^{32}P-ATP. When the full length MEKK1 protein was immunoprecipitated by the 12CA5 antibody it had measurable autophosphorylation and activity towards SEK1. When MEKK1 was immunoprecipitated with the COOH-terminal 95-012 antibody, a stronger SEK1 phosphorylation signal was detected. Since the full length MEKK1 protein and fragments C and D are immunoprecipitated with similar efficiency, the increased phosphorylation of SEK1 was due to the presence of fragments C and D in the immunoprecipitates. This phosphorylation was reduced in the presence of CrmA. In the presence of p35, phosphorylation of SEK1 reached the same level of phosphorylation observed when the 12CA5 antibody was used, that is the basal level of phosphorylation induced by the full length MEKK1. Phosphorylation of fragments C and D was also detected in 95-012 immunoprecipitates. This phosphorylation was reduced by CrmA and almost completely abolished by p35, as expected from the effect of these inhibitors on the generation of fragments C and D. In summary, there is a strong correlation between MEKK1-induced apoptosis and the generation of MEKK1-derived cleavage products that have a stronger kinase activity than the full length protein. This suggests that proteolysis of MEKK1 is involved in the cell death response.

<u>p35 inhibited cleavage occurs at position Asp^{874} in the mouse MEKK1 protein.</u>

The p35-inhibited cleavage of MEKK1 generates a COOH-terminal fragment of about 90 kDa and a NH_2-terminal fragment of about 110 kDa, indicating that the

cleavage occurs between residues 820-900. Two tetrapeptide sequences that are found in this region of MEKK1 closely resemble the CPP32 cleavage site, DEVD (SEQ ID NO: 12) (Nicholson *et al.*, 1995). These sequences are ^{857}DEVE^{860} (SEQ ID NO: 7) and ^{871}DTVD^{874} (SEQ ID NO: 8) (see Fig. 4). The proteases inhibited by p35 have been shown to be cysteine proteases cleaving after the aspartic acid residue in the fourth position of the consensus cleavage sequence (Nicholson *et al.*, 1995; Howard *et al.*, 1991) and, therefore only the DTVD (SEQ ID NO: 8) sequence should be a cleavage site for the CPP32-like protease. Two mutants were generated that have either the DEVE (SEQ ID NO: 7) or the DTVD (SEQ ID NO: 8) sequence replaced with alanine residues (see Fig. 4). These mutants were transfected into HEK293 cells and the presence of MEKK1 and MEKK1-derived fragments were detected by immunoblot analysis using three MEKK1-specific antibodies. When transfected into HEK293 cells, the DEVE→A mutant, like the wild-type protein, was cleaved into fragments A, B, C and D. In contrast, the DTVD→A mutant was only cleaved into fragments A and D. Thus, fragments B and C are not generated in cells expressing the DTVD→A mutant or in cells expressing MEKK1 and p35. This indicates that the p35-inhibited cleavage occurs at position Asp^{874} in the mouse MEKK1 sequence.

The kinase activity of the mutants expressed in HEK293 cells was determined. Immunoprecipitating full length 196 kDa MEKK1 or mutant MEKK1 proteins with the 12CA5 antibody resulted in similar SEK1 phosphorylating activities. However, when the antibodies directed towards the COOH-terminus of the protein were used, SEK1 phosphorylating activity was reduced in DTVD→A expressing cells as compared to the activity found in wild-type or DEVE→A expressing cells. The reduced kinase activity was comparable to the basal SEK1 phosphorylating activity observed when the full length proteins were immunoprecipitated. Thus, the mutant DTVD→A MEKK1 protein has a low but measurable kinase activity towards SEK1 because fragment C is not generated. The same result was observed when the cleavage of MEKK1 into fragments B and C was inhibited by p35 expression.

Based on the results described above, Fig. 5 describes a model of the MEKK1 cleavage events occurring in transfected cells. In this model, overexpression of MEKK1 induces deregulated cleavage events generating two sets of fragments (A and D; B and C). Fragment C encoding the catalytic domain of MEKK1 has a stronger kinase activity than the full length protein. Proteases of the ICE/FLICE family are responsible for the cleavage of MEKK1 into fragments B and C because this cleavage can be inhibited by p35 and CrmA. Mutagenesis experiments revealed that the cleavage site generating fragments B and C is DTVD^{874} (SEQ ID NO: 8). Fragment C can be further processed into a smaller polypeptide (fragment D) which may be rapidly degraded. It is possible

that the proteolytic activity which generates fragment D is part of a regulatory mechanism involved in the termination of the response induced by cleavage of MEKK1 into the active fragment C.

The DTVD→A mutant has a reduced ability to promote DNA fragmentation in HEK293 cells.

It was next determined whether the DTVD→A mutant induces DNA fragmentation when expressed in HEK293 cells. Expression of the DEVE→A mutant or the wild-type MEKK1 protein induced DNA fragmentation. In contrast, cells expressing the DTVD→A mutant MEKK1 protein showed little DNA fragmentation. Quantitation of the response revealed that the number of DTVD→A expressing cells that showed some DNA fragmentation was reduced by 65% compared to the cells transfected with wild-type MEKK1 or the DEVE→A mutant. This indicates that cleavage of MEKK1 into fragments B and C is required to induce cell death.

p35 inhibits ΔMEKK1-induced apoptosis.

The 37 kDa kinase domain of MEKK1 (ΔMEKK1) is a strong inducer of apoptosis (Lassignal Johnson *et al.*, 1996; Xia *et al.*, 1995). Since p35 inhibits programmed cell death induced by most, if not all, apoptotic stimuli (Clem *et al.*, 1996), it was also determined whether this inhibitor could also block ΔMEKK1-induced apoptosis. ΔMEKK1 induced DNA fragmentation when expressed in HEK293 cells. This effect was inhibited by co-expression of p35. Quantitation showed that 40% of cells expressing ΔMEKK1 showed DNA breaks; co-expression of p35 and ΔMEKK1 reduced this number to 10%. The number of ΔMEKK1-expressing cells appeared to be increased when p35 was present, suggesting that less cell death occurred when ΔMEKK1 and p35 were co-expressed. Even if the co-transfected cells showed less DNA fragmentation compared to the cells transfected with ΔMEKK1 alone, they were clearly affected by the expression of ΔMEKK1 and were rounded and most showed some membrane blebbing. This differed from the effect of p35 in full length MEKK1-transfected cells, where the inhibitor appeared to better protect the cells from DNA fragmentation and obvious morphological changes, the predicted result if cleavage of MEKK1 results in the release of an activated kinase domain. These results indicate that p35 inhibits at least two steps in the pathway leading to MEKK1-induced apoptosis, the cleavage of MEKK1 into an active kinase fragment and events downstream of the MEKK1 cleavage that most likely involves a protease step that is influenced by MEKK1.

Activation of the ERK and the JNK pathways is not correlated with MEKK1-induced DNA fragmentation.

MEKK1 activates the ERK and JNK pathways (Xu *et al.*, 1996). Since activation of the JNK pathway has been proposed to induce apoptosis (Verheij *et al.*, 1996), it was next determined whether inhibitory mutants of JNK1 or JNK2 (JNK1-APF and JNK2-APF, respectively) could prevent MEKK1-induced DNA fragmentation. While JNK1-APF had no protective effect, JNK2-APF slightly (by about 30%) reduced the number of MEKK1-expressing apoptotic cells. The competitive inhibitory JNK mutants had no effect on the generation of any cleavage products, indicating that the JNK2-APF-mediated inhibition of MEKK1-induced DNA fragmentation is not related to the cleavage of MEKK1. Activation of ERK2 or the JNKs by MEKK1 was unaffected by the co-expression of JNK1-APF, JNK2-APF, p35 or CrmA. When specific JNK isoforms were immunoprecipitated, only JNK1-APF and JNK2-APF partially inhibited JNK1 and JNK2 activity, respectively. The partial inhibition may be due to cross-reactivity of the antibodies used (Gupta *et al.*, 1996). The DEVE→A and DTVD→A mutants activated JNK to the same level as wild type MEKK1. Transfection of MEKK1 in HEK293 cells did not activate the p38 kinase. Cumulatively, these results show that in conditions where MEKK1-induced DNA fragmentation is inhibited (*i.e.* when p35 is cotransfected with MEKK1 or when the DTVD→A mutant is expressed), the ERK and the JNK pathways are still activated to an extent similar to that found in MEKK1-transfected cells. This indicates that neither the ERK nor the JNK pathways are sufficient to promote or inhibit the cell death pathway induced by cleavage of MEKK1.

UV irradiation of HEK293 cells induces a rapid phosphorylation and subsequent cleavage of the endogenous MEKK1protein.

To determine the relevance of these findings in a more physiological situation, the regulation of endogenous MEKK1 in response to UV irradiation, a stress stimulus that induces an apoptotic response, was examined. In HEK293 cells, three different antisera directed at the mouse MEKK1 protein recognized the 196 kDa MEKK1 protein. Several additional nonspecific immunoreactive protein bands were also detected. When cells were treated with UV irradiation (100 J/m^2) and incubated for 24 hours in low serum media, the full length MEKK1 protein was no longer detected. Since, we have determined that the half-life of MEKK1 is greater than 24 hours, this result indicates that UV induces a cleavage of the MEKK1 protein. UV irradiation also induced the appearance of new immunoreactive species, the majority of which have molecular weights ranging from about 100 kDa to about 120 kDa. These polypeptides appear thus

to be MEKK1-derived fragments generated following MEKK1 proteolysis. The results indicate that UV induces cleavage of the endogenous MEKK1 protein in HEK293 cells.

A time course was performed to determine the effects of UV irradiation on the endogenous MEKK1 protein, activation of the JNK pathway and the extent of apoptosis resulting from the exposure of the cells to a stress stimulus. 15 min after UV irradiation, an MEKK1 species is generated that was upward gel-shifted compared to the MEKK1 species detected before exposure to UV irradiation. One hour after irradiation, most of the full length MEKK1 protein was upward gel-shifted. Eight hours after irradiation, the amount of the gel-shifted MEKK1 started to decrease and 20 hours after UV treatment only a trace amount of full length MEKK1 was detected. The MEKK1 fragment detected by the 96-001 (NH2) antibody was barely seen in the control condition. After 1 hour, however, there was a clear increase in the production of the MEKK1 fragment which reached a maximum 8 hours after UV irradiation. In MEKK1-transfected cells, both the shifted and non-shifted forms of full length MEKK1 were detected. To determine whether the upward gel shift of MEKK1 was due to phosphorylation, lysates of MEKK1-transfected cells were immunoprecipitated with the 12CA5 antibody and incubated with or without protein phosphatase 2A (PP-2A). Phosphatase treatment converted the upper, gel-shifted, form to the lower band, demonstrating that the gel-shift was a phosphorylation-dependent event. To determine whether phosphorylation of MEKK1 was required for its activity, the ability of immunoprecipitated MEKK1 to phosphorylate its substrate SEK1 was assessed after pretreatment with PP-2A. Immunoprecipitates treated with phosphatase did not phosphorylate SEK1. Thus, phosphorylation of MEKK1 is required for its activation. These results show that UV irradiation induced a rapid phosphorylation of full length MEKK1 followed by its cleavage into fragments. The extent of JNK activation after UV irradiation paralleled the extent of MEKK1 phosphorylation, consistent with the fact that MEKK1 is an upstream regulator of the JNK pathway. Apoptosis, as assessed by morphological changes of the nucleus, started to be detected 8 hours after UV irradiation and was most apparent after 20 hours.

Cleavage of MEKK1 can be mediated by different stress stimuli.

Several genotoxic stress stimuli were applied to HEK293 cells and their effect on the MEKK1 protein was assessed. UV irradiation, cisplatin, etoposide and mitomycin C induced the loss of full length MEKK1 and the appearance of a lower molecular weight fragment derived from MEKK1. While there was no full length MEKK1 protein remaining after UV and cisplatin treatments, a small amount of upward gel-shifted full length MEKK1 was detected in etoposide and mitomycin C-treated cells. This indicates

that chemicals capable of forming DNA adducts, induce the phosphorylation of MEKK1 before its cleavage. These results indicate that the cleavage of MEKK1 may be the activation step leading to apoptosis in a number of stress conditions.

An emerging theme for the cellular commitment to apoptosis involves the activation of specific proteases and the regulation of signal transduction pathways, but the integration of these two regulatory processes in the apoptotic response has not been clearly defined. The role of ICE/FLICE proteases being involved in the apoptotic response is unequivocal (Fraser and Evan, 1996). Loss or inhibition of these enzyme activities can inhibit apoptosis (Los *et al.*, 1995; Darmon and Bleackley, 1996). The notion that signal transduction pathways, specifically those involving the c-Jun kinases and p38 kinases, has developed based on correlative biochemical analysis and gene transfection experiments. An inhibitory mutant of SEK1 (c-Jun kinase kinase) was demonstrated to block ceramide-induced apoptosis in different cell types (Verheij *et al.*, 1996). Similarly, it was shown that a dominant negative c-Jun mutant could block apoptosis of serum-deprived neuronal cells (Xia *et al.*, 1995). Activated mutants of p38 and its immediate upstream regulatory kinase MKK3 was shown to enhance an apoptotic response of PC12 cells to serum deprivation (Xia *et al.*, 1995). The ERK pathway has been shown to have a protective response against an apoptotic stimulus in a few cell types (Xia *et al.*, 1995; Gardner and Johnson, 1996). However, discordance for a role of c-Jun kinases and p38 kinases in mediating apoptosis also exists. For example, MEKK1 mediated apoptosis was shown to be independent of c-Jun kinase activation (Lassignal Johnson *et al.*, 1996). A similar separation of c-Jun kinase activation and apoptosis was observed with the TNF receptor (Liu *et al.*, 1996b).

In this example, it is demonstrated that the JNK pathway is clearly not sufficient to induce the apoptosis mediated by MEKK1. Numerous other examples exist where c-Jun kinase and p38 are activated in response to a stimulus but apoptosis is not observed (Su *et al.*, 1994; Sumimoto *et al.*, 1994; Tsubata *et al.*, 1993). What is however evolving from these studies is that the integration of several different signals, including the regulation of MAP kinase pathways (Xia *et al.*, 1995; Gardner and Johnson, 1996), can contribute to the decision of a cell to commit to apoptosis. Just as with growth and differentiation a series of checkpoints must be overcome before a cell commits itself to death. The needed commitment appears to be activation of the ICE/FLICE protease cascade; activation of c-Jun kinase or p38 pathways may be insufficient by themselves but may enhance or prevent the apoptotic response resulting from an external stimulus such as a genotoxic agent or cytokine.

MEKK1-mediated apoptosis requires both kinase activity and proteolytic cleavage.

We have shown previously that the kinase activity of MEKK1 is required for its apoptotic activity, because the kinase-inactive (MEKK1 is unable to promote apoptosis (Lassignal Johnson *et al.*, 1996). Here it is shown that there is a tight integration of kinase and protease activities in the MEKK1-induced apoptotic pathway. Proteases are required for MEKK1-induced apoptosis at at least two levels in the transduction pathway. The first level corresponds to the cleavage of MEKK1 at position 874 in the mouse MEKK1 sequence. When this cleavage is prevented by the p35 baculovirus protein or when a cleavage-resistant MEKK1 mutant is used, apoptosis is strongly impaired. Proteases of the ICE family of proteases are required for this cleavage to occur, since the viral inhibitors CrmA and p35 inhibit the cleavage. It is indeed likely that CPP32 or a CPP32-like enzyme directly cleaves MEKK1 at position 874, because the recognition site for the protease in the mouse MEKK1 is DTVD, a sequence that closely resembles the DEVD recognition site of the CPP32 substrate poly (ADP-ribose) polymerase (Nicholson *et al.*, 1995). The sequence in the rat MEKK1 sequence that corresponds to the murine DTVD cleavage recognition site is DTLD (Xu *et al.*, 1996); indicating that the cleavage site is conserved between the mouse and the rat MEKK1 proteins and further supports its importance in MEKK1 function. ICE-like proteases are also required at a second step that is downstream of the cleavage of MEKK1 because p35 inhibits the apoptosis induced by the kinase domain of MEKK1.

Fig. 6 shows a model defining the involvement of MEKK1 in apoptosis. The 196 kDa MEKK1 protein can be activated by many extracellular inputs including tyrosine kinase encoded growth factor receptors, G protein-coupled receptors (Avdi *et al.*, 1996) and cellular stresses. Activation of MEKK1 correlates with its phosphorylation. It is unclear at present if MEKK1 phosphorylation involves autophosphorylation or additional kinases. Activated MEKK1 independent of its proteolysis is capable of regulating the c-Jun kinase pathway and may also regulate the ERK pathway. Both of these pathways can stimulate anti-apoptotic responses. Stimulation of the JNK pathway can lead to NFκB activation which is a strong inhibitor of apoptosis (Baeuerle and Baltimore, 1996) and activation of the ERK pathway has been shown to protect cells from apoptosis (Xia *et al.*, 1995; Gardner and Johnson, 1996). With an appropriate protease activation MEKK1 is cleaved to generate a 91 kDa activated kinase domain that has substrates that contribute to driving the cell to apoptosis. Downstream of these phosphorylation events are additional protease substrates that are predicted to be either phosphoproteins or proteins whose activity is regulated by phosphoproteins and which are involved in regulating apoptosis. Bcl-2, for example, would be such a phosphoprotein candidate (Gajewski and Thompson, 1996).

Proteolysis of MEKK1 generates an activated fragment with altered cellular distribution.

It has been demonstrated that the endogenous MEKK1 in resting cells is localized in a post-Golgi vesicular compartment. The punctate cytoplasmic staining of MEKK1 can be seen in non-transfected cells. Upon appropriate cellular stimulation by a growth factor such as EGF MEKK1 is translocated to the plasma membrane. When MEKK1 is overexpressed it is activated and becomes proteolyzed. When MEKK1 is proteolyzed the catalytic domain behaves as a soluble cytoplasmic protein that is no longer sequestered on vesicle-like structures or the plasma membrane. Cleavage of MEKK1 may also change the specificity and activity of the kinase. In vitro kinase assays have indeed revealed that the kinase activity of the cleaved MEKK1 towards SEK1 is increased compared to the full length MEKK1. Thus, the 91 kDa kinase fragment of MEKK1 has a different subcellular distribution from the 196 kDa holo-MEKK1 which may allow it to phosphorylate a different set of substrates.

Genotoxic stress: A balance between rescue and suicide using MEKK1 as a switch.

The results show that DNA damaging chemicals such as cisplatin, etoposide and mitomycin C in addition to UV irradiation induce a phosphorylation correlated with activation of MEKK1. The time course for UV irradiation-induced c-Jun kinase activation closely paralleled that for MEKK1 phosphorylation, consistent with MEKK1 being an upstream regulator of this pathway. Thus, UV irradiation induces a rapid phosphorylation and activation of MEKK1 and c-Jun kinase. The rapid c-Jun kinase response could actually contribute to a protective response against cell death. This has been proposed for the action of CD40 in protecting B cells from antigen crosslinking-induced apoptosis (Sumimoto *et al.*, 1994; Tsubata *et al.*, 1993) and methyl methane sulfonate-induced 3T3 cell apoptosis (Liu *et al.*, 1996a). The activation of NFκB in response to stresses including UV irradiation and genotoxic chemicals would also be a protective response (Baeuerle and Baltimore, 1996); MEKK1 has been shown to be involved in the activation of NFκB (Hirano *et al.*, 1996).

If the stress challenge to the cell is too great a protease cascade is activated involving the ICE/FLICE enzymes (Fraser and Evan, 1996). The data indicate that one substrate for CPP32-like proteases is MEKK1. The time course of MEKK1 proteolysis is slower than its activation; cleavage of MEKK1 releases the 91 kDa kinase domain with new subcellular localization and the ability to activate effectors of apoptosis.

These findings suggest MEKK1 can function as a switch point, regulated by a proteolytic event controlled by ICE/FLICE proteases, that determines cell fate in response to a stress stimulus. Before cleavage MEKK1 induces rescue mechanisms and

after cleavage MEKK1 triggers apoptosis. The cleavage of MEKK1 may thus occur when the cell has failed to successfully repair itself. The cleaved MEKK1 then triggers apoptosis which leads to the elimination of the cell.

The above example defines MEKK1 as a protease substrate that when activated and cleaved stimulates an apoptotic response. The proteolytic cleavage of MEKK1 defines the mechanism to generate a protein kinase whose activity is sufficient to induce apoptosis. In the context of cancer therapy, the finding that the activation and cleavage of MEKK1 occurs in response to genotoxic agents is particularly important. For example, expression of MEKK1 is capable of killing by apoptosis cells that have both p53 alleles mutated. Hence, the activation and cleavage of MEKK1 is an apoptotic pathway that does not require a functional p53 and stimulation of these events could enhance the killing of many different tumors. Manipulating the activation of MEKK1 and its cleavage by proteases, with the use of drugs for example, could increase the killing of tumor cells to genotoxic agents. This is consistent with the finding that low level expression of MEKK1 potentiated the apoptotic response to low doses of UV irradiation and cisplatin.

Citations for Publications Referred to in this Example:

An and Dou (1996) Cancer Res. *56*, 438-442; Avdi *et al.* (1996) J. Biol. Chem. *271*, 33598-33606; Baeuerle and Baltimore (1996) Cell *87*, 13-20; Beidler *et al.* (1996) J. Biol. Chem. *270*, 16526-16528; Blank *et al.* (1996) J. Biol. Chem. *271*, 5361-5368. Canman and Kastan (1996) Nature *384*, 213-214; Cartier *et al.* (1994) J. Virol. *68*, 7728-7737; Casciola-Rosen *et al.* (1994) J. Biol. Chem. *269*, 30757-30760; Casciola-Rosen *et al.* (1995) J. Exp. Med. *182*, 1625-1634; Clem *et al.* (1996) Death Differ. *3*, 9-16. Cryns *et al.* (1996) J. Biol. Chem. *271*, 31277-31282; Darmon *et al.* (1996) J. Biol. Chem. *271*, 21699-21702; Dérijard *et al.* (1994) Cell *76*, 1025-1037; Emoto *et al.* (1995) EMBO J. *14*, 6148-6156; Esolen *et al.* (1995) J. Virol. *69*, 3955-3958; Fraser and Evan (1996) Cell *85*, 781-784; Gajewski and Thompson (1996) Cell *87*, 589-592; Gardner and Johnson (1996) J. Biol. Chem. *271*, 14560-14566; Gupta *et al.* (1996) EMBO J. *15*, 2760-2770; Hibi *et al.* (1993) Genes Develop. *7*, 2135-2148; Hinshaw *et al.* (1994) J. Virol. *68*, 3667-3673; Hirano *et al.* (1996) J. Biol. Chem. *271*, 13234-13238; Howard (1991) J. Immunol. *147*, 2964-2969.

Juo *et al.* (1997) Mol. Cell. Biol. *17*, 24-35; Kallunki *et al.* (1994) Genes Develop. *8*, 2996-3007; Kägi *et al.* (1994) Science *265*, 528-530; Lange-Carter *et al.* (1993) Science *260*, 315-319; Lange-Carter and Johnson (1994) Science *265*, 1458-1461; Lassignal Johnson *et al.* (1996) J. Biol. Chem. *271*, 3229-3237; Lazebnik *et al.* (1994) Nature *371*,

346-347; Lazebnik *et al.* (1995) Proc. Natl. Acad. Sci. U. S. A. *92*, 9042-9046; Liu *et al.* (1996a) Nature *384*, 273-276; Liu *et al.* (1996b) Cell *87*, 565-576; Los *et al.* (1995) Nature *375*, 81-83.

Lowin *et al.* (1994) Nature *370*, 650-652; Na *et al.* (1996) J. Biol. Chem. *271*, 11209-11213.

Nicholson *et al.* (1995) Nature *376*, 37-43; Nicoletti *et al.* (1991) J. Immunol. Methods *139*, 271-279; Orth *et al.* (1996) J. Biol. Chem. *271*, 20977-20980; Park *et al.* (1996) J. Biol. Chem. *271*, 21898-21905; Pickup *et al.* (1986) Proc. Natn. Acad. Sci. U. S. A. *83*, 7698-7702; Steller (1995) Science *267*, 1445-1449; Su *et al.* (1994) Cell *77*, 727-736; Sumimoto *et al.* (1994) J. Immunol. *153*, 2488-2496; Terai *et al.* (1991) J. Clin. Invest. *87*, 1710-1715; Tsubata *et al.* (1993) Nature *364*, 645-648; Tyler *et al.* (1995) J. Virol. *69*, 6972-6979; Vandenabeele *et al.* (1995) Trends Cell Biol. *5*, 392-399; Verheij *et al.* (1996) *et al.* Nature *380*, 75-79; Wang *et al.* (1996) EMBO J. *15*, 1012-1020; Widmann *et al.* (1995) Biochem. J. *310*, 203-214; Williams and Smith (1993) Cell *74*, 777-779; Xia *et al.* (1995) Science *270*, 1326-1331; and Xu *et al.* (1996) Proc. Natl. Acad. Sci. U. S. A. *93*, 5291-5295.

The foregoing description of the invention has been presented for purposes of illustration and description. Further, the description is not intended to limit the invention to the form disclosed herein. Consequently, variations and modifications commensurate with the above teachings, and the skill or knowledge in the relevant art are within the scope of the present invention. The preferred embodiment described herein above is further intended to explain the best mode known of practicing the invention and to enable others skilled in the art to utilize the invention in various embodiments and with various modifications required by their particular applications or uses of the invention. It is intended that the appended claims be construed to include alternate embodiments to the extent permitted by the prior art. The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference.

We Claim:

1. An isolated nucleic acid molecule selected from the group consisting of:

a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:3 or a complement thereof;

b) a nucleic acid molecule comprising a fragment of at least 100 contiguous nucleotides of a nucleic acid comprising the nucleotide sequence of SEQ ID NO:3 or a complement thereof;

c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:4;

d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:4, wherein the fragment comprises at least 15 contiguous amino acid residues of the amino acid sequence of SEQ ID NO:4;

e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:4, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:3 under stringent conditions; and

f) a nucleic acid molecule which is antisense to the coding strand of a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:3.

2. The nucleic acid molecule of claim 1 further comprising vector nucleic acid sequences.

3. The nucleic acid molecule of claim 1 further comprising nucleic acid sequences encoding a heterologous polypeptide.

4. A host cell which contains the nucleic acid molecule of claim 1.

5. An isolated nucleic acid molecule selected from the group consisting of:

a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:5 or a complement thereof;

b) a nucleic acid molecule comprising a fragment of at least 100 contiguous nucleotides of a nucleic acid comprising the nucleotide sequence of SEQ ID NO:5 or a complement thereof;

c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:6;

d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:6, wherein the fragment comprises at least 15 contiguous amino acid residues of the amino acid sequence of SEQ ID NO:6;

e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:6, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:5 under stringent conditions; and

f) a nucleic acid molecule which is antisense to the coding strand of a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:5.

6. The nucleic acid molecule of claim 5 further comprising vector nucleic acid sequences.

7. The nucleic acid molecule of claim 5 further comprising nucleic acid sequences encoding a heterologous polypeptide.

8. A host cell which contains the nucleic acid molecule of claim 5.

9. An isolated polypeptide selected from the group consisting of:

a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:4, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:4;

b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:4, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:3 under stringent conditions;

c) a polypeptide which is encoded by the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:3; and

d) a polypeptide comprising the amino acid sequence of SEQ ID NO:4.

10. The fusion protein comprising the polypeptide of claim 9 operatively linked to heterologous amino acid sequences.

11. An antibody which selectively binds to a polypeptide of claim 9.

12. A method for producing a polypeptide selected from the group consisting of:

a) a polypeptide comprising the amino acid sequence of SEQ ID NO:4;

b) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:4, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:4; and

c) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:4, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:3 under stringent conditions;

comprising culturing the host cell of claim 4 under conditions in which the nucleic acid molecule is expressed.

13. An isolated polypeptide selected from the group consisting of:

a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:6, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:6;

b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:6, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:5 under stringent conditions;

c) a polypeptide which is encoded by the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:5; and

d) a polypeptide comprising the amino acid sequence of SEQ ID NO:6.

14. The fusion protein comprising the polypeptide of claim 13 operatively linked to heterologous amino acid sequences.

15. An antibody which selectively binds to a polypeptide of claim 13.

16. A method for producing a polypeptide selected from the group consisting of:

a) a polypeptide comprising the amino acid sequence of SEQ ID NO:6;

b) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:6, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:6; and

c) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:6, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:5 under stringent conditions;

comprising culturing the host cell of claim 8 under conditions in which the nucleic acid molecule is expressed.

17. A method for detecting the presence of a MEKK1 polypeptide in a sample comprising:

a) contacting the sample with a compound which selectively binds to the polypeptide; and

b) determining whether the compound binds to the polypeptide in the sample to thereby detect the presence of a MEKK1 polypeptide in the sample.

18. A kit comprising a compound which selectively binds to a MEKK1 polypeptide and instructions for use.

19. A method for detecting the presence of a MEKK1 nucleic acid molecule in a sample comprising:

a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and

b) determining whether the nucleic acid probe or primer binds to a nucleic acid molecule in the sample to thereby detect the presence of a MEKK1 nucleic acid molecule in the sample.

20. A kit comprising a compound which selectively hybridizes to a MEKK1 nucleic acid molecule and instructions for use.

21. A method for detecting the presence of a biological activity of a MEKK1 polypeptide in a sample comprising:

a) contacting the sample with an agent capable of detecting MEKK1 activity; and

b) determining the presence of MEKK1 activity in the sample.

22. A method for modulating MEKK1 activity comprising contacting a cell with an agent that modulates MEKK1 activity such that MEKK1 activity in the cell is modulated.

23. The method of claim 22 wherein the agent is selected form the group consisting of an antibody that specifically binds to the MEKK1 protein and a nucleic acid molecule having a nucleotide sequence which is antisense to the coding strand of a MEKK1 mRNA of MEKK1 gene.

24. A method to treat a subject having a disorder characterized by aberrant MEKK1 protein or nucleic acid expression or activity comprising administering an agent which is a MEKK1 modulator to the subject such that MEKK1 protein or nucleic acid expression or activity is modulated.

25. A method for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a MEKK1 protein; (ii) mis-regulation of said gene; and (iii) aberrant post-translational modification of a MEKK1 protein, wherein a wild-type form of said gene encodes an protein with a MEKK1 activity

26. An isolated active fragment of an MEKK1 protein consisting of an amino acid sequence having at least 75% homology to an amino acid sequence consisting of about amino acids 875-1493 of SEQ ID NO:4, wherein said active fragment mediates apoptosis.

27. The active fragment of claim 26, which consists of an amino acid sequence having at least 85% homology to an amino acid sequence consisting of about amino acids 875-1493 of SEQ ID NO:4.

28. The active fragment of claim 26, which consists of an amino acid sequence having at least 95% homology to an amino acid sequence consisting of about amino acids 875-1493 of Figure 9.

29. The active fragment of claim 26, which is a mouse MEKK1 active fragment.

30. The active fragment of claim 26, which is a human MEKK1 active fragment.

31. The active fragment of claim 26, which is a rat MEKK1 active fragment.

32. The active fragment of claim 26, which consists of about amino acids 875-1493 of SEQ ID NO:4.

33. The active fragment of claim 26, which consists of about amino acids 685-1303 of SEQ ID NO:6.

34. An isolated protease-resistant MEKK1 protein comprising an amino acid sequence having at least 75% homology to the amino acid sequence of SEQ ID NO:4, wherein at least one amino acid equivalent to amino acids 871-874 of SEQ ID NO:4 is substituted such that the MEKK1 protein is resistant to proteolysis by a caspase after amino acid 874.

35. The MEKK1 protein of claim 34, wherein at least one amino acid equivalent to amino acids 871-874 of SEQ ID NO:4 is substituted with an alanine residue.

36. The MEKK1 protein of claim 34, wherein each amino acid equivalent to amino acids 871-874 of SEQ ID NO:4 is substituted with an alanine residue.

37. The MEKK1 protein of claim 34, which has at least 85% homology to the amino acid sequence of SEQ ID NO:4.

38. The MEKK1 protein of claim 34, which has at least 95% homology to the amino acid sequence of SEQ ID NO:4.

39. The MEKK1 protein of claim 34, which is a mouse MEKK1 protein.

40. The MEKK1 protein of claim 34, which is a human MEKK1 protein.

41. The MEKK protein of claim 40 consisting of amino acids 685-1303 of SEQ ID NO:6.

42. The MEKK1 protein of claim 34, which is a rat MEKK1 protein.

43. An isolated nucleic acid molecule consisting of a nucleotide sequence having at least 75% homology to a nucleotide sequence consisting of about nucleotides 2637-4493 of SEQ ID NO:3, wherein said nucleic acid molecule encodes an active fragment of MEKK1 that mediates apoptosis.

44. The nucleic acid molecule of claim 43, which consists of a nucleotide sequence having at least 85% homology to a nucleotide sequence consisting of about nucleotides 2637-4493 of SEQ ID NO:3.

45. The nucleic acid molecule of claim 43, which consists of a nucleotide sequence having at least 95% homology to a nucleotide sequence consisting of about nucleotides 2637-4493 of SEQ ID NO:3.

46. The nucleic acid molecule of claim 43, which encodes an active fragment of a mouse MEKK1.

46. The nucleic acid molecule of claim 43, which encodes an active fragment of a human MEKK1.

48. The nucleic acid molecule of claim 43, which encodes an active fragment of a rat MEKK1.

49. The nucleic acid molecule of claim 43, which consists of about nucleotides 2637-4493 of SEQ ID NO:3, or a nucleotide sequence that, due to the degeneracy of the genetic code, encodes the same amino acid sequence as about nucleotides 2637-4493 of SEQ ID NO:3.

50. The nucleic acid molecule of claim 43, which consists of nucleotides 2637-4493 of SEQ ID NO:3, or a nucleotide sequence that, due to the degeneracy of the genetic code, encodes the same amino acid sequence as nucleotides 2637-4493 of SEQ ID NO:3.

51. The nucleic acid molecule of claim 43, which consists of nucleotides 2052-3908 of SEQ ID NO:5, or a nucleotide sequence that, due to the degeneracy of the genetic code, encodes the same amino acid sequence as nucleotides 2052-3908 of SEQ ID NO:5.

52. An isolated nucleic acid molecule encoding a protease-resistant MEKK1 protein, wherein the protease resistant MEKK1 protein comprises an amino acid sequence having at least 75% homology to the amino acid sequence of SEQ ID NO:4 and at least one codon of the nucleic acid molecule encoding an amino acid equivalent to at least one of amino acids 871-874 of SEQ ID NO:4 is mutated such the encoded MEKK1 protein is resistant to proteolysis by a caspase after an amino acid equivalent to amino acid 871-874 of SEQ ID NO:4.

53. The nucleic acid molecule of claim 52, wherein the MEKK1 protein comprises an amino acid sequence having at least 85% homology to the amino acid sequence of SEQ ID NO:4.

54. The nucleic acid molecule of claim 52, wherein the MEKK1 protein comprises an amino acid sequence having at least 95% homology to the amino acid sequence of SEQ ID NO:4.

55. The nucleic acid molecule of claim 52, which encodes a protease-resistant mouse MEKK1 protein.

56. The nucleic acid molecule of claim 52, which encodes a protease-resistant human MEKK1 protein.

57. The nucleic acid molecule of claim 52, which encodes a protease-resistant rat MEKK1 protein.

58. An expression vector comprising the nucleic acid molecule of claim 43.

59. An expression vector comprising the nucleic acid molecule of claim 52.

60. A host cell containing the expression vector of claim 58.

61. A host cell containing the expression vector of claim 59.

62. An isolated nucleic acid molecule encoding a protease-resistant MEKK1 protein, wherein the protease resistant MEKK1 protein comprises the amino acid sequence of SEQ ID NO:6 and at least one codon of the nucleic acid molecule encoding an amino acid equivalent to at least one of amino acids 681-684 of SEQ ID NO:6 is mutated such the encoded MEKK1 protein is resistant to proteolysis by a caspase after an amino acid equivalent to amino acid 681-684 of SEQ ID NO:6.

63. A method of stimulating apoptosis in a cell comprising introducing into the cell an expression vector encoding a MEKK1 active fragment such that MEKK1 active fragment is produced in the cell and apoptosis is stimulated.

64. A method of inhibiting apoptosis in a cell comprising introducing into the cell an expression vector encoding a protease-resistant MEKK1 protein such that protease-resistant MEKK1 protein is produced in the cell and apoptosis is inhibited.

65. A method of generating an MEKK1 active fragment *in vitro*, comprising:

contacting an MEKK1 protein *in vitro* with a caspase protease under proteolysis conditions; and

allowing the caspase protease to cleave the MEKK1 protein such that an MEKK1 active fragment is generated.

66. A method of identifying a compound that modulates the apoptotic activity of an MEKK1 active fragment, comprising:

providing an indicator cell that comprises a MEKK1 active fragment;

contacting the indicator cell with a test compound; and

determining the effect of the test compound on the apoptotic activity of the MEKK1 active fragment in the indicator cell to thereby identify a compound that modulates the apoptotic activity of the MEKK1 active fragment.

67. A method of identifying a compound that modulates the proteolytic cleavage of an MEKK1 protein by a caspase protease, comprising:

providing a reaction mixture that comprises an MEKK1 protein and a caspase protease;

contacting the reaction mixture with a test compound; and

determining the effect of the test compound on proteolytic cleavage of the MEKK1 protein by the caspase protease to thereby identify a compound that modulates the proteolytic cleavage of an MEKK1 protein by a caspase protease.

```
LOCUS       HMEKK1 NUC   3911 BP DS-
        1 CGGCCTGGAA GCACGAGTGG TTGGAAAGGA GAAATAGGCG AGGGCCTGTG GTGGTAAAAC
       61 CAATCCCAGT TAAAGGAGAT GGATCTGAAA TGAATCACTT AGCAGCTGAG TCTCCAGGAG
      121 AGGTCCAGGC AAGTGCGGCT TCACCAGCTT CCAAAGGCCG ACGCAGTCCT TCTCCTGGCA
      181 ACTCCCCATC AGGTCGCACA GTGAAATCAG AATCTCCAGG AGTAAGGAGA AAAAGAGTTT
      241 CCCCAGTGCC TTTTCAGAGT GGCAGAATCA CACCACCCCG AAGAGCCCCT TCACCAGATG
      301 GCTTCTCACC ATATAGCCCT GAGGAAACAA ACCGCCGTGT TAACAAAGTG ATGCGGGCCA
      361 GACTGTACTT ACTGCAGCAG ATAGGGCCTA ACTCTTTCCT GATTGGAGGA GACAGCCCAG
      421 ACAATAAATA CCGGGTGTTT ATTGGGCCTC AGAACTGCAG CTGTGCACGT GGAACATTCT
      481 GTATTCATCT GCTATTTGTG ATGCTCCGGG TGTTTCAACT AGAACCTTCA GACCCAATGT
      541 TATGGAGAAA AACTTTAAAG AATTTTGAGG TTGAGAGTTT GTTCCAGAAA TATCACAGTA
      601 GGCGTAGCTC AAGGATCAAA GCTCCATCTC GTAACACCAT CCAGAAGTTT GTTTCACGCA
      661 TGTCAAATTC TCATACATTG TCATCATCTA GTACTTCTAC ATCTAGTTCA GTAAACAGCA
      721 TAAAGGATGA AGAGGAACAG ATGTGTCCTA TTTGCTTGTT GGGCATGCTT GATGAAGAAA
      781 GTCTTACAGT GTGTGAAGAC GGCTGCAGGA ACAAGCTGCA CCACCACTGC ATGTCAATTT
      841 GGGCAGAAGA GTGTAGAAGA AATAGAGAAC CTTTAATATG TCCCCTTTGT AGATCTAAGT
      901 GGAGATCTCA TGATTTCTAC AGCCACGAGT TGTCAAGTCC TGTGGATTCC CCTTCTTCCC
      961 TCAGAGCTGC ACAGCAGCAA ACCGTACAGC AGCAGCCTTT GGCTGGATCA CGAAGGAATC
     1021 AAGAGAGCAA TTTTAACCTT ACTCATTATG GAACTCAGCA AATCCCTCCT GCTTACAAAG
     1081 ATTTAGCTGA GCCATGGATT CAGGTGTTTG GAATGGAACT CGTTGGCTGC TTATTCTCTA
     1141 GAAACTGGAA CGTAAGGGAA ATGGCCCTTA GGCGTCTTTC CCACGACGTT AGTGGGGCCC
     1201 TGTTGTTGGC AAACGGGGAG AGCACTGGAA ACTCTGGAGG CGGCAGTGGG GGCAGCTTAA
     1261 GCGCGGGAGC GGCCAGCGGG TCCTCCCAGC CCAGCATCTC AGGGGATGTG GTGGAGGCGT
     1321 GCTGCAGTGT CCTGTCTATA GTCTGCGCTG ACCCTGTCTA CAAAGTGTAC GTTGCTGCTT
     1381 TAAAAACATT GAGAGCCATG CTGGTATACA CTCCTTGCCA CAGTCTGGCA GAAAGAATCA
     1441 AACTTCAGAG ACTCCTCCGG CCAGTTGTAG ACACTATCCT TGTCAAGTGT GCAGATGCCA
     1501 ACAGCCGCAC GAGTCAGCTG TCCATATCTA CAGTGCTGGA ACTCTGCAAT GGCCAAGCAG
     1561 GAAAGCTGGC GGTTGGGAGA GAAATACTTA AAGCTGGGTC CATCGGGGTT GGTGGTGTCG
     1621 ATTACGTCTT AAGTTGTATC CTTGGAAACC AAGCTGAATC AAACAACTGG CAAGAACTGC
     1681 TGGGTCGCCT CTGTCTTATA GACAGGTTGC TGTTGGAATT TCCTGCTGAA TTCTATCCTC
     1741 ATATTGTCAG TACTGATGTC TCACAAGCTG AGCCTGTTGA AATCAGGTAC AAGAAGCTGC
     1801 TCTCCCTCTT AACCTTTGCC TTGCAATCCA TTGACAATTC CCACTCGATG GTTGGCAAGC
     1861 TCTCTCGGAG GATATATCTG AGCTCTGCCA GGATGGTGAC CGCAGTGCCC GCTGTGTTTT
     1921 CCAAGCTGGT AACCATGCTT AATGCTTCTG GCTCCACCCA CTTCACCAGG ATGCGCCGGC
     1981 GTCTGATGGC TATCGCGGAT GAGGTAGAAA TTGCCGAGGT CATCCAGCTG GGTGTGGAGG
     2041 ACACTGTGGA TGGGCATCAG GACAGCTTAC AGGCGCTGGC CCCCGCCAGC TGTCTAGAAA
     2101 ACAGCTCCCT TGAGCACACA GTCCATAGAG AGAAAACTGG AAAAGGACTA AGTGCTACGA
     2161 GACTGAGTGC CAGCTCGGAG GACATTTCTG ACAGACTGGC CGGCGTCTCT GTAGGACTTC
     2221 CCAGCTCAAC AACAACAGAA CAACCAAAGC CAGCGGTTCA AACAAAAGGC AGACCCCACA
     2281 GTCAGTGTTT GAACTCCTCC CCTTTGTCTC ATGCTCAATT AATGTTCCCA GCACCATCAG
     2341 CCCCTTGTTC CTCTGCCCCG TCTGTCCCAG ATATTTCTAA GCACAGACCC CAGGCATTTG
     2401 TTCCCTGCAA AATACCTTCC GCATCTCCTC AGACACAGCG CAAGTTCTCT CTACAATTCC
     2461 AGAGGAACTG CTCTGAACAC CGAGACTCAG ACCAGCTCTC CCCAGTCTTC ACTCAGTCAA
     2521 GACCCCCACC CTCCAGTAAC ATACACAGGC CAAAGCCATC CCGACCCGTT CCGGGCAGTA
     2581 CAAGCAAACT AGGGGACGCC ACAAAAAGTA GCATGACACT TGATCTGGGC AGTGCTTCCA
     2641 GGTGTGACGA CAGCTTTGGC GGCGGCGGCA ACAGTGGCAA CGCCGTCATA CCCAGCGACG
     2701 AGACAGTGTT CACGCCGGTG GAGGACAAGT GCAGGTTAGA TGTGAACACC GAGCTCAACT
     2761 CCAGCATCGA GGACCTTCTT GAAGCATCCA TGCCTTCAAG TGACACGACA GTCACTTTCA
     2821 AGTCCGAAGT CGCCGTCCTC TCTCCGGAAA AGGCCGAAAA TGACGACACC TACAAAGACG
     2881 ACGTCAATCA TAATCAAAAG TGCAAAGAAA AGATGGAAGC TGAAGAGGAG GAGGCTTTAG
     2941 CGATCGCCAT GGCGATGTCA GCGTCTCAGG ATGCCCTCCC CATCGTCCCT CAGCTGCAGG
     3001 TGGAAAATGG AGAAGATATT ATCATCATTC AGCAGGACAC ACCAGAAACT CTTCCAGGAC
     3061 ATACCAAAGC GAAACAGCCT TACAGAGAAG ACGCTGAGTG GCTGAAAGGC CAGCAGATAG
     3121 GCCTCGGAGC ATTTTCTTCT TGTTATCAGG CTCAAGATGT GGGAACTGGA ACTTTAATGG
     3181 CTGTTAAACA GGTGACTTAT GTCAGAAACA CATCTTCTGA GCAAGAAGAA GTAGTAGAAG
     3241 CACTAAGAGA AGAGATAAGA ATGATGAGCC ATCTGAATCA TCCAAACATC ATTAGGATGT
     3301 TGGGAGCCAC GTGTGAGAAG AGCAATTACA ATCTCTTCAT TGAATGGATG GCAGGGGGAT
```

FIGURE 1A

```
3361 CGGTGGCTCA TTTGCTGAGT AAATATGGAG CCTTCAAAGA ATCAGTAGTT ATTAACTACA
3421 CTGAACAGTT ACTCCGTGGC CTTTCGTATC TCCATGAGAA CCAGATCATT CACAGAGATG
3481 TCAAAGGTGC CAATTTGCTC ATTGACAGCA CCGGTCAGAG GCTGAGAATT GCAGACTTTG
3541 GAGCTGCAGC CAGGTTGGCA TCAAAAGGAA CTGGTGCAGG AGAGTTTCAG GGACAATTAC
3601 TGGGGACAAT TGCATTCATG GCGCCTGAGG TCCTAAGAGG TCAGCAGTAT GGTAGGAGCT
3661 GTGATGTATG GAGTGTTGGC TGCGCCATTA TAGAAATGGC TTGTGCAAAA CCACCTTGGA
3721 ATGCAGAAAA ACACTCCAAT CATCTCGCCT TGATATTTAA GATTGCTAGC GCAACTACTG
3781 CACCGTCCAT CCCGTCACAC CTGTCCCCTG GTTTACGAGA TGTGGCTCTT CGTTGTTTAG
3841 AACTTCAGCC TCAGGACCGG CCTCCGTCAA GAGAGCTGCT GAAACATCCG GTCTTCCGTA
3901 CCACGTGGTA G
```

FIGURE 1B

Mouse MEKK1 cDNA

```
          10          20          30          40
     *    *    *    *    *    *    *    *    *
GCC CGC GAG AGA AAA TGG CGG CGG CGG CGG GCG ATC GCG CCT CGT
CGG GCG CTC TCT TTT ACC GCC GCC GCC GCC CGC TAG CGC GGA GCA

     50          60          70          80          90
     *    *    *    *    *    *    *    *    *    *
CGT CGG GAT TCC CGG GCG CCG CGG CGG CGA GTC CCG AGG CGG GCG
GCA GCC CTA AGG GCC CGC GGC GCC GCC GCT CAG GGC TCC GCC CGC

          100         110         120         130
     *    *    *    *    *    *    *    *    *    *
GCG GCG GCG GAG GAG GAG GAG CTC TCC AGG GAA GCG GCG CGC CCG
CGC CGC CGC CTC CTC CTC CTC GAG AGG TCC CTT CGC CGC GCG GGC

     140         150         160         170         180
     *    *    *    *    *    *    *    *    *    *
CAG CGG GCG CGG CGG GGC TGC TGC GGG AGC CTG GCA GCG CGG GCC
GTC GCC CGC GCC GCC CCG ACG ACG CCC TCG GAC CGT CGC GCC CGG

          190         200         210         220
     *    *    *    *    *    *    *    *    *    *
GCG AGC GCG CGG ACT GGC GGC GGC GGC AGC TGC GCA AAG TGC GGA
CGC TCG CGC GCC TGA CCG CCG CCG CCG TCG ACG CGT TTC ACG CCT

     230         240         250         260         270
     *    *    *    *    *    *    *    *    *    *
GTG TGG AGC TGG ACC AGC TGC CGG AGC AGC CGC TCT TCC TCG CCG
CAC ACC TCG ACC TGG TCG ACG GCC TCG TCG GCG AGA AGG AGC GGC

          280         290         300         310
     *    *    *    *    *    *    *    *    *    *
CCG CCT CGC CGC CCT GCC CAT CTA CTT CCC CGT CGC CGG AGC CCG
GGC GGA GCG GCG GGA CGG GTA GAT GAA GGG GCA GCG GCC TCG GGC

     320         330         340         350         360
     *    *    *    *    *    *    *    *    *    *
CGG ACG CGG CTG CAG GAG CGA GTC GCT TCC AGC CCG CGG CGG GAC
GCC TGC GCC GAC GTC CTC GCT CAG CGA AGG TCG GGC GCC GCC CTG

          370         380         390         400
     *    *    *    *    *    *    *    *    *    *
CGC CAC CCC CGG GAG CGG CGA GTC GCT GCG GCT CCC ACT CTG CCG
GCG GTG GGG GCC CTC GCC GCT CAG CGA CGC CGA GCG TGA GAC GGC

     410         420         430         440         450
     *    *    *    *    *    *    *    *    *    *
AGC TGG CGG CCG CGC GGG ACA GCG GCG CCC GGA GCC CCG CGG GGG
TCG ACC GCC GGC GCG CCC TGT CGC CGC GGG CCT CGG GGC GCC CCC

          460         470         480         490
     *    *    *    *    *    *    *    *    *    *
CCG AGC CGC CCT CTG CAG CGG CCC CCT CCG GTC GAG AGA TGG AGA
GGC TCG GCG GGA GAC GTC GCC GGG GGA GGC CAG CTC TCT ACC TCT

     500         510         520         530         540
     *    *    *    *    *    *    *    *    *    *
```

FIGURE 2A

Mouse MEKK1 cDNA

```
ATA AAG AAA CCC TCA AAG GAC TGC ACA AGA TGG AGG ATC GCC CGG
TAT TTC TTT GGG AGT TTC CTG ACG TGT TCT ACC TCC TAG CGG GCC

          550         560         570         580
     *    *    *    *    *    *    *    *    *
AGG AGA GAA TGA TCC GGG AGA AGC TCA AGG CGA CCT GTA TGC CGG
TCC TCT CTT ACT AGG CCC TCT TCG AGT TCC GCT GGA CAT ACG GCC

   590         600         610         620         630
     *    *    *    *    *    *    *    *    *    *
CCT GGA AGC ACG AGT GGT TGG AGA GGA GGA ACA GGA GAG GCC CTG
GGA CCT TCG TGC TCA CCA ACC TCT CCT CCT TGT CCT CTC CGG GAC

          640         650         660         670
     *    *    *    *    *    *    *    *    *
TGG TGG TGA AGC CAA TCC CTA TTA AAG GAG ATG GAT CTG AAG TGA
ACC ACC ACT TCG GTT AGG GAT AAT TTC CTC TAC CTA GAC TTC ACT

   680         690         700         710         720
     *    *    *    *    *    *    *    *    *
ATA ACT TGG CAG CTG AGC CCC AGG GAG AGG GCC AGG CAG GTT CCG
TAT TGA ACC GTC GAC TCG GGG TCC CTC TCC CGG TCC GTC CAA GGC

          730         740         750         760
     *    *    *    *    *    *    *    *    *
CTG CAC CAG CCC CCA AGG GCC GAC GAA GCC CAT CTC CTG GCA GCT
GAC GTG GTC GGG GGT TCC CGG CTG CTT CGG GTA GAG GAC CGT CGA

   770         780         790         800         810
     *    *    *    *    *    *    *    *    *    *
CTC CGT CAG GGC GCT CGG TGA AGC CGG AAT CCC CAG GAG TAA GAC
GAG GCA GTC CCG CGA GCC ACT TCG GCC TTA GGG GTC CTC ATT CTG

          820         830         840         850
     *    *    *    *    *    *    *    *    *
GGA AAC GAG TGT CCC CGG TGC CTT TCC AGA GTG GCA GAA TCA CAC
CCT TTG CTC ACA GGG GCC ACG GAA AGG TCT CAC CGT CTT AGT GTG

   860         870         880         890         900
     *    *    *    *    *    *    *    *    *    *
CAC CCC GAA GAG CCC CAT CAC CGG ATG GCT TCT CCC CGT ACA GCC
GTG GGG CTT CTC GGG GTA GTG GCC TAC CGA AGA GGG GCA TGT CGG

          910         920         930         940
     *    *    *    *    *    *    *    *    *
CAG AGG AGA CGA GCC GCC GCG TGA ACA AAG TGA TGA GAG CCA GGC
GTC TCC TCT GCT CGG CGG CGC ACT TGT TTC ACT ACT CTC GGT CCG

   950         960         970         980         990
     *    *    *    *    *    *    *    *    *    *
TGT ACC TGC TGC AGC AGA TAG GAC CCA ACT CTT TCC TGA TTG GAG
ACA TGG ACG ACG TCG TCT ATC CTG GGT TGA GAA AGG ACT AAC CTC

         1000        1010        1020        1030
     *    *    *    *    *    *    *    *    *
GAG ACA GTC CAG ACA ATA AAT ACC GGG TGT TTA TTG GGC CAC AGA
CTC TGT CAG GTC TGT TAT TTA TGG CCC ACA AAT AAC CCG GTG TCT
```

FIGURE 2B

Mouse MEKK1 cDNA

```
1040        1050        1060        1070        1080
ACT GCA GCT GTG GGC GTG GAG CAT TCT GTA TTC ACC TCT TGT TTG
TGA CGT CGA CAC CCG CAC CTC GTA AGA CAT AAG TGG AGA ACA AAC

      1090        1100        1110        1120
TCA TGC TCC GGG TGT TTC AGC TAG AAC CCT CTG ACC CCA TGT TAT
AGT ACG AGG CCC ACA AAG TCG ATC TTG GGA GAC TGG GGT ACA ATA

1130        1140        1150        1160        1170
GGA GAA AAA CTT TAA AAA ATT TCG AGG TTG AGA GTT TGT TCC AGA
CCT CTT TTT GAA ATT TTT TAA AGC TCC AAC TCT CAA ACA AGG TCT

      1180        1190        1200        1210
AAT ACC ACA GTA GGC GTA GCT CGA GAA TCA AAG CTC CAT CCC GGA
TTA TGG TGT CAT CCG CAT CGA GCT CTT AGT TTC GAG GTA GGG CCT

1220        1230        1240        1250        1260
ACA CCA TCC AGA AGT TTG TGT CAC GCA TGT CAA ATT CTC ACA CAC
TGT GGT AGG TCT TCA AAC ACA GTG CGT ACA GTT TAA GAG TGT GTG

      1270        1280        1290        1300
TGT CAT CGT CTA GCA CAT CCA CAT CTA GTT CAG AAA ACA GCA TCA
ACA GTA GCA GAT CGT GTA GGT GTA GAT CAA GTC TTT TGT CGT AGT

1310        1320        1330        1340        1350
AGG ATG AAG AGG AGC AGA TGT GTC CCA TCT GCT TGC TGG GCA TGC
TCC TAC TTC TCC TCG TCT ACA CAG GGT AGA CGA ACG ACC CGT ACG

      1360        1370        1380        1390
TGG ATG AGG AGA GCC TGA CTG TGT GTG AAG ATG GCT GCA GGA ACA
ACC TAC TCC TCT CGG ACT GAC ACA CAC TTC TAC CGA CGT CCT TGT

1400        1410        1420        1430        1440
AGC TGC ACC ACC ATT GCA TGT CCA TCT GGG CGG AAG AGT GTA GAA
TCG ACG TGG TGG TAA CGT ACA GGT AGA CCC GCC TTC TCA CAT CTT

      1450        1460        1470        1480
GAA ATA GAG AGC CTT TAA TAT GTC CCC TTT GTA GAT CTA AGT GCA
CTT TAT CTC TCG GAA ATT ATA CAG GGG AAA CAT CTA GAT TCA CGT

1490        1500        1510        1520        1530
GAT CCC ATG ACT TCT ACA GCC ATG AGT TAT CAA GCC CCG TGG AGT
CTA GGG TAC TGA AGA TGT CGG TAC TCA ATA GTT CGG GGC ACC TCA

      1540        1550        1560        1570
```

FIGURE 2C

Mouse MEKK1 cDNA

```
                                                                    *
CCC CCG CCT CCC TGC GAG CTG TCC AGC AGC CAT CCT CCC CGC AGC
GGG GGC GGA GGG ACG CTC GAC AGG TCG TCG GTA GGA GGG GCG TCG

  1580          1590          1600          1610          1620
    *             *             *             *             *
AGC CCG TGG CCG GAT CAC AGC GGA GGA ATC AGC AGA GCA GTT TTA
TCG GGC ACC GGC CTA GTG TCG CCT CCT TAG TCG TCT CGT CAA AAT

          1630          1640          1650          1660
            *             *             *             *
ACC TTA CTC ATT TTG GAA CCC AGC AGA TTC CTT CCG CTT ACA AAG
TGG AAT GAG TAA AAC CTT GGG TCG TCT AAG GAA GGC GAA TGT TTC

  1670          1680          1690          1700          1710
    *             *             *             *             *
ATT TGG CCG AGC CAT GGA TTC AGG TGT TTG GAA TGG AAC TCG TTG
TAA ACC GGC TCG GTA CCT AAG TCC ACA AAC CTT ACC TTG AGC AAC

          1720          1730          1740          1750
            *             *             *             *
GCT GCT TAT TCT CTA GAA ACT GGA ACG TAA GGG AAA TGG CCC TTA
CGA CGA ATA AGA GAT CTT TGA CCT TGC ATT CCC TTT ACC GGG AAT

  1760          1770          1780          1790          1800
    *             *             *             *             *
GGC GTC TTT CCC ACG ACG TTA GTG GGG CCC TGT TGT TGG CAA ACG
CCG CAG AAA GGG TGC TGC AAT CAC CCC GGG ACA ACA ACC GTT TGC

          1810          1820          1830          1840
            *             *             *             *
GGG AGA GCA CTG GAA ACT CTG GAG GCG GCA GTG GGG GCA GCT TAA
CCC TCT CGT GAC CTT TGA GAC CTC CGC CGT CAC CCC CGT CGA ATT

  1850          1860          1870          1880          1890
    *             *             *             *             *
GCG CGG GAG CGG CCA GCG GGT CCT CCC AGC CCA GCA TCT CAG GGG
CGC GCC CTC GCC GGT CGC CCA GGA GGG TCG GGT CGT AGA GTC CCC

          1900          1910          1920          1930
            *             *             *             *
ATG TGG TGG AGG CGT GCT GCA GTG TCC TGT CTA TAG TCT GCG CTG
TAC ACC ACC TCC GCA CGA CGT CAC AGG ACA GAT ATC AGA CGC GAC

  1940          1950          1960          1970          1980
    *             *             *             *             *
ACC CTG TCT ACA AAG TGT ACG TTG CTG CTT TAA AAA CAT TGA GAG
TGG GAC AGA TGT TTC ACA TGC AAC GAC GAA ATT TTT GTA ACT CTC

          1990          2000          2010          2020
            *             *             *             *
CCA TGC TGG TAT ACA CTC CTT GCC ACA GTC TGG CAG AAA GAA TCA
GGT ACG ACC ATA TGT GAG GAA CGG TGT CAG ACC GTC TTT CTT AGT

  2030          2040          2050          2060          2070
    *             *             *             *             *
AAC TTC AGA GAC TCC TCC GGC CAG TTG TAG ACA CTA TCC TTG TCA
```

FIGURE 2D

Mouse MEKK1 cDNA

```
TTG AAG TCT CTG AGG AGG CCG GTC AAC ATC TGT GAT AGG AAC AGT

        2080        2090        2100        2110
    *       *   *       *   *       *   *       *   *
AGT GTG CAG ATG CCA ACA GCC GCA CGA GTC AGC TGT CCA TAT CTA
TCA CAC GTC TAC GGT TGT CGG CGT GCT CAG TCG ACA GGT ATA GAT

  2120        2130        2140        2150        2160
    *       *   *       *   *       *   *       *   *
CAG TGC TGG AAC TCT GCA AGG GCC AAG CAG GAG AGC TGG CGG TTG
GTC ACG ACC TTG AGA CGT TCC CGG TTC GTC CTC TCG ACC GCC AAC

        2170        2180        2190        2200
    *       *   *       *   *       *   *       *   *
GGA GAG AAA TAC TTA AAG CTG GGT CCA TCG GGG TTG GTG GTG TCG
CCT CTC TTT ATG AAT TTC GAC CCA GGT AGC CCC AAC CAC CAC AGC

  2210        2220        2230        2240        2250
    *       *   *       *   *       *   *       *   *
ATT ACG TCT TAA GTT GTA TCC TTG GAA ACC AAG CTG AAT CAA ACA
TAA TGC AGA ATT CAA CAT AGG AAC CTT TGG TTC GAC TTA GTT TGT

        2260        2270        2280        2290
    *       *   *       *   *       *   *       *   *
ACT GGC AAG AAC TGC TGG GTC GCC TCT GTC TTA TAG ACA GGT TGC
TGA CCG TTC TTG ACG ACC CAG CGG AGA CAG AAT ATC TGT CCA ACG

  2300        2310        2320        2330        2340
    *       *   *       *   *       *   *       *   *
TGT TGG AAT TTC CTG CTG AAT TCT ATC CTC ATA TTG TCA GTA CTG
ACA ACC TTA AAG GAC GAC TTA AGA TAG GAG TAT AAC AGT CAT GAC

        2350        2360        2370        2380
    *       *   *       *   *       *   *       *   *
ATG TCT CAC AAG CTG AGC CTG TTG AAA TCA GGT ACA AGA AGC TGC
TAC AGA GTG TTC GAC TCG GAC AAC TTT AGT CCA TGT TCT TCG ACG

  2390        2400        2410        2420        2430
    *       *   *       *   *       *   *       *   *
TCT CCC TCT TAA CCT TTG CCT TGC AAT CCA TTG ACA ATT CCC ACT
AGA GGG AGA ATT GGA AAC GGA ACG TTA GGT AAC TGT TAA GGG TGA

        2440        2450        2460        2470
    *       *   *       *   *       *   *       *   *
CGA TGG TTG GCA AGC TCT CTC GGA GGA TAT ATC TGA GCT CTG CCA
GCT ACC AAC CGT TCG AGA GAG CCT CCT ATA TAG ACT CGA GAC GGT

  2480        2490        2500        2510        2520
    *       *   *       *   *       *   *       *   *
GGA TGG TGA CCG CAG TGC CCG CTG TGT TTT CCA AGC TGG TAA CCA
CCT ACC ACT GGC GTC ACG GGC GAC ACA AAA GGT TCG ACC ATT GGT

        2530        2540        2550        2560
    *       *   *       *   *       *   *       *   *
TGC TTA ATG CTT CTG GCT CCA CCC ACT TCA CCA GGA TGC GCC GGC
ACG AAT TAC GAA GAC CGA GGT GGG TGA AGT GGT CCT ACG CGG CCG
```

FIGURE 2E

Mouse MEKK1 cDNA

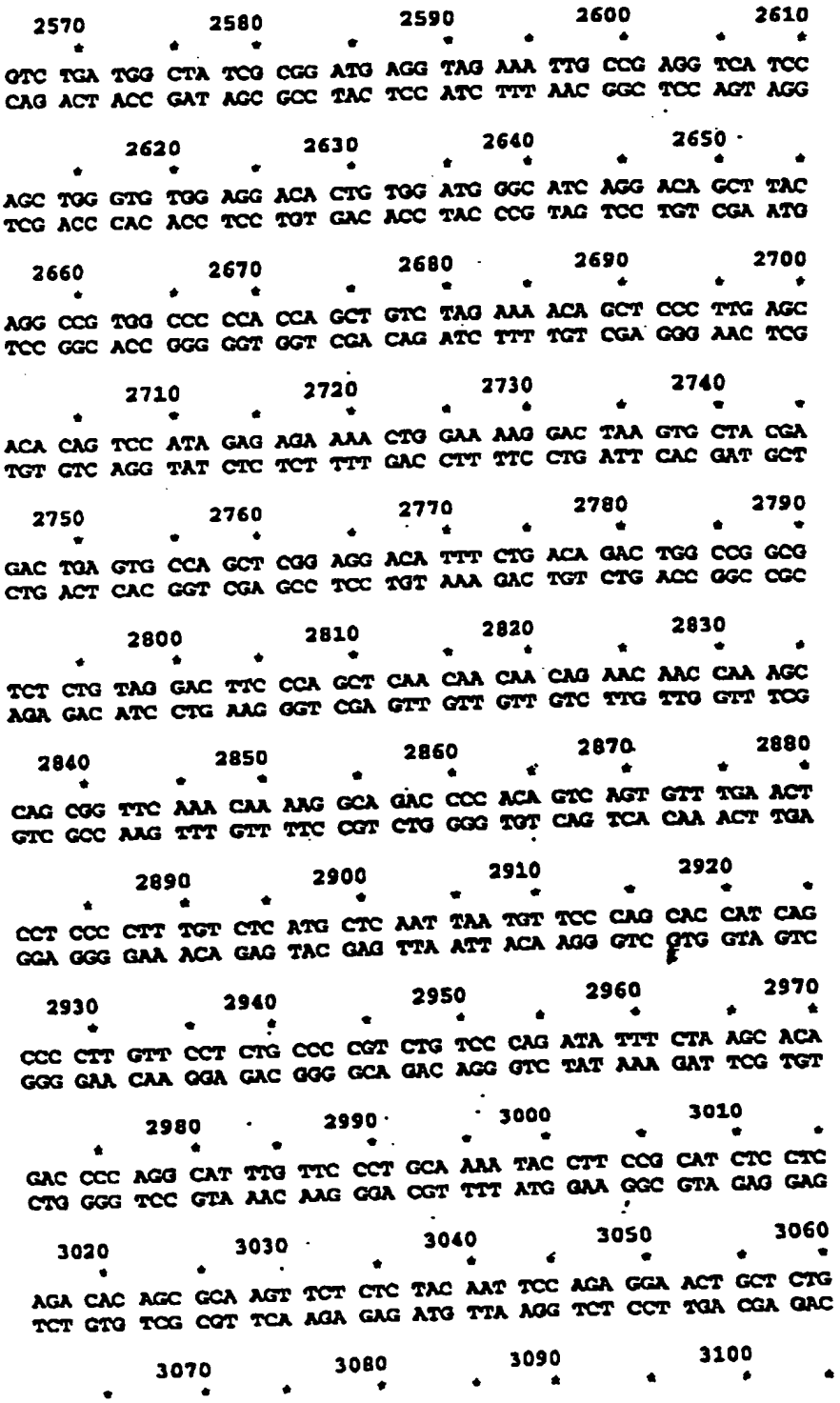


FIGURE 2F

Mouse MEKK1 cDNA

```
AAC ACC GAG ACT CAG ACC AGC TCT CCC CAG TCT TCA CTC AGT CAA
TTG TGG CTC TGA GTC TGG TCG AGA GGG GTC AGA AGT GAG TCA GTT

  3110        3120        3130        3140        3150
    *           *           *           *           *
GAC CCC CAC CCT CCA GTA ACA TAC ACA GGC CAA AGC CAT CCC GAC
CTG GGG GTG GGA GGT CAT TGT ATG TGT CCG GTT TCG GTA GGG CTG

        3160        3170        3180        3190
    *           *           *           *           *
CCG TTC CGG GCA GTA CAA GCA AAC TAG GGG ACG CCA CAA AAA GTA
GGC AAG GCC CGT CAT GTT CGT TTG ATC CCC TGC GGT GTT TTT CAT

  3200        3210        3220        3230        3240
    *           *           *           *           *
GCA TGA CAC TTG ATC TGG GCA GTG CTT CCA GGT GTG ACG ACA GCT
CGT ACT GTG AAC TAG ACC CGT CAC GAA GGT CCA CAC TGC TGT CGA

        3250        3260        3270        3280
    *           *           *           *           *
TTG GCG GCG GCG GCA ACA GTG GCA ACG CCG TCA TAC CCA GCG ACG
AAC CGC CGC CGC CGT TGT CAC CGT TGC GGC AGT ATG GGT CGC TGC

  3290        3300        3310        3320        3330
    *           *           *           *           *
AGA CAG TGT TCA CGC CGG TGG AGG ACA AGT GCA GGT TAG ATG TGA
TCT GTC ACA AGT GCG GCC ACC TCC TGT TCA CGT CCA ATC TAC ACT

        3340        3350        3360        3370
    *           *           *           *           *
ACA CCG AGC TCA ACT CCA GCA TCG AGG ACC TTC TTG AAG CAT CCA
TGT GGC TCG AGT TGA GGT CGT AGC TCC TGG AAG AAC TTC GTA GGT

  3380        3390        3400        3410        3420
    *           *           *           *           *
TGC CTT CAA GTG ACA CGA CAG TCA CTT TCA AGT CCG AAG TCG CCG
ACG GAA GTT CAC TGT GCT GTC AGT GAA AGT TCA GGC TTC AGC GGC

        3430        3440        3450        3460
    *           *           *           *           *
TCC TCT CTC CGG AAA AGG CCG AAA ATG ACG ACA CCT ACA AAG ACG
AGG AGA GAG GCC TTT TCC GGC TTT TAC TGC TGT GGA TGT TTC TGC

  3470        3480        3490        3500        3510
    *           *           *           *           *
ACG TCA ATC ATA ATC AAA AGT GCA AAG AAA AGA TGG AAG CTG AAG
TGC AGT TAG TAT TAG TTT TCA CGT TTC TTT TCT ACC TTC GAC TTC

        3520        3530        3540        3550
    *           *           *           *           *
AGG AGG AGG CTT TAG CGA TCG CCA TGG CGA TGT CAG CGT CTC AGG
TCC TCC TCC GAA ATC GCT AGC GGT ACC GCT ACA GTC GCA GAG TCC

  3560        3570        3580        3590        3600
    *           *           *           *           *
ATG CCC TCC CCA TCG TCC CTC AGC TGC AGG TGG AAA ATG GAG AAG
TAC GGG AGG GGT AGC AGG GAG TCG ACG TCC ACC TTT TAC CTC TTC
```

FIGURE 2G

Mouse MEKK1 cDNA

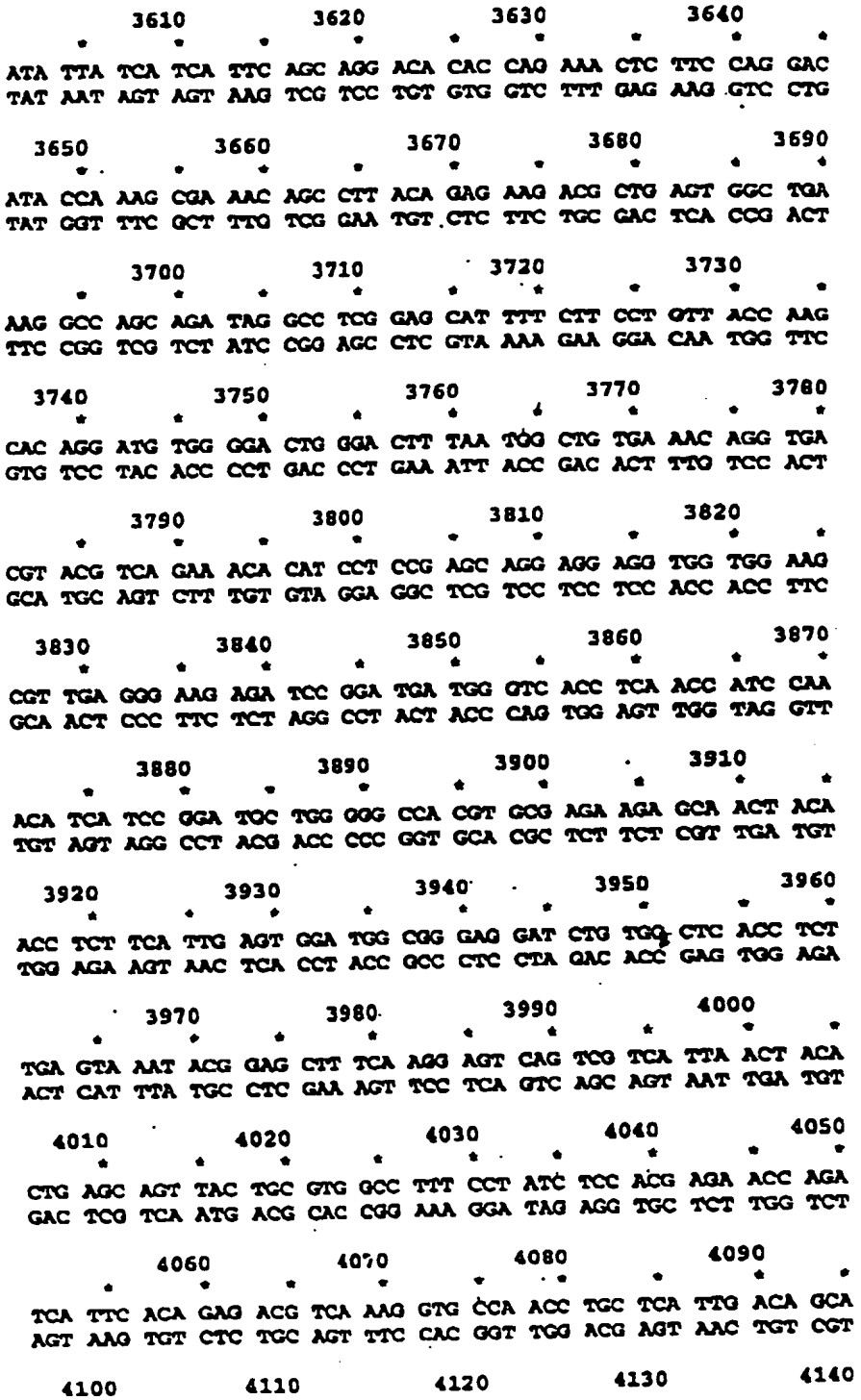


FIGURE 2H

Mouse MEKK1 cDNA

```
                                                                    *
CCG GTC AGA GGC TGA GAA TTG CAG ACT TTG GAG CTG CTG CCA GGT
GGC CAG TCT CCG ACT CTT AAC GTC TGA AAC CTC GAC GAC GGT CCA

          4150          4160          4170          4180
     *     *     *     *     *     *     *     *     *
TGG CAT CAA AAG GAA CCG GTG CAG GAG AGT TCC AGG GAC AGT TAC
ACC GTA GTT TTC CTT GGC CAC GTC CTC TCA AGG TCC CTG TCA ATG

  4190          4200          4210          4220          4230
    *     *     *     *     *     *     *     *     *     *
TGG GGA CAA TTG CAT TCA TGG CGC CTG AGG TCC TAA GAG GTC AGC
ACC CCT GTT AAC GTA AGT ACC GCG GAC TCC AGG ATT CTC CAG TCG

          4240          4250          4260          4270
     *     *     *     *     *     *     *     *     *
AGT ATG GTA GGA GCT GTG ATG TAT GGA GTG TTG GCT GCG CCA TTA
TCA TAC CAT CCT CGA CAC TAC ATA CCT CAC AAC CGA CGC GGT AAT

  4280          4290          4300          4310          4320
    *     *     *     *     *     *     *     *     *     *
TAG AAA TGG CTT GTG CAA AAC CAC CTT GGA ATG CAG AAA AAC ACT
ATC TTT ACC GAA CAC GTT TTG GTG GAA CCT TAC GTC TTT TTG TGA

          4330          4340          4350          4360
     *     *     *     *     *     *     *     *     *
CCA ATC ATC TCG CCT TGA TAT TTA AGA TTG CTA GCG CAA CTA CTG
GGT TAG TAG AGC GGA ACT ATA AAT TCT AAC GAT CGC GTT GAT GAC

  4370          4380          4390          4400          4410
    *     *     *     *     *     *     *     *     *     *
CAC CGT CCA TCC CGT CAC ACC TGT CCC CGG GTC TGC GCG ACG TGG
GTG GCA GGT AGG GCA GTG TGG ACA GGG GCC CAG ACG CGC TGC ACC

          4420          4430          4440          4450
     *     *     *     *     *     *     *     *     *     *
CCG TGC GCT GCT TAG AAC TTC AGC CTC AGG ACC GGC CTC CGT CCA
GGC ACG CGA CGA ATC TTG AAG TCG GAG TCC TGG CCG GAG GCA GGT

  4460          4470          4480          4490          4500
    *     *     *     *     *     *     *     *     *     *
GAG AGC TGC TGA AAC ATC CGG TCT TCC GTA CCA CGT GGT AGT TAA
CTC TCG ACG ACT TTG TAG GCC AGA AGG CAT GGT GCA CCA TCA ATT

          4510          4520          4530          4540
     *     *     *     *     *     *     *     *     *     *
TTG TTC AGA TCA GCT CTA ATG GAG ACA GGA TAT GCA ACC GGG AGA
AAC AAG TCT AGT CGA GAT TAC CTC TGT CCT ATA CGT TGG CCC TCT

  4550          4560          4570          4580          4590
    *     *     *     *     *     *     *     *     *     *
GAG AAA AGA GAA CTT GTG GGC GAC CAT GCC GCT AAC CGC AGC CCT
CTC TTT TCT CTT GAA CAC CCG CTG GTA CGG CGA TTG GCG TCG GGA

          4600          4610          4620          4630
     *     *     *     *     *     *     *     *     *     *
CAC GCC ACT GAA CAG CCA GAA ACG GGG CCA GCG GGG AAC CGT ACC
```

FIGURE 2I

Mouse MEKK1 cDNA

```
GTG CGG TGA CTT GTC GGT CTT TGC CCC GGT CGC CCC TTG GCA TGG

  4640        4650        4660        4670        4680
    *     *     *     *     *     *     *     *     *
TAA GCA TGT GAT TGA CAA ATC ATG ACC TGT ACC TAA GCT CGA TAT
ATT CGT ACA CTA ACT GTT TAG TAC TGG ACA TGG ATT CGA GCT ATA

        4690        4700        4710        4720
    *     *     *     *     *     *     *     *     *
GCA GAC ATC TAC AGC TCG TGC AGG AAC TGC ACA CCG TGC CTT TCA
CGT CTG TAG ATG TCG AGC ACG TCC TTG ACG TGT GGC ACG GAA AGT

  4730        4740        4750        4760        4770
    *     *     *     *     *     *     *     *     *
CAG GAC TGG CTC TGG GGG ACC AGG AAG GCG ATG GAG TTT GCA TGA
GTC CTG ACC GAG ACC CCC TGG TCC TTC CGC TAC CTC AAA CGT ACT

        4780        4790        4800        4810
    *     *     *     *     *     *     *     *     *
CTA AAG AAC AGA AGC ATA AAT TTA TTT TTG GAG CAC TTT TTC AGC
GAT TTC TTG TCT TCG TAT TTA AAT AAA AAC CTC GTG AAA AAG TCG

  4820        4830        4840        4850        4860
    *     *     *     *     *     *     *     *     *
TAA TCA GTA TTA CCA TGT ACA TCA ACA TGC CCG CCA CAT TTC AAA
ATT AGT CAT AAT GGT ACA TGT AGT TGT ACG GGC GGT GTA AAG TTT

        4870        4880        4890        4900
    *     *     *     *     *     *     *     *     *
CTC AGA CTG TCC CAG ATG TCA AGA TCC ACT GTG TTT GAG TTT GTT
GAG TCT GAC AGG GTC TAC AGT TCT AGG TGA CAC AAA CTC AAA CAA

  4910        4920        4930        4940        4950
    *     *     *     *     *     *     *     *     *
TGC AGT TCC CTC AGC TTG CTG GTA ATT GTG GTG TTT TGT TTT CGA
ACG TCA AGG GAG TCG AAC GAC CAT TAA CAC CAC AAA ACA AAA GCT

        4960        4970        4980        4990
    *     *     *     *     *     *     *     *     *
TGC AAA TGT GAT GTA ATA TTC TTA TTT TCT TTG GAT CAA AGC TGG
ACG TTT ACA CTA CAT TAT AAG AAT AAA AGA AAC CTA GTT TCG ACC

  5000        5010        5020        5030        5040
    *     *     *     *     *     *     *     *     *
ACT GAA AAT TGT ACT GTG TAA TTA TTT TTG TGT TTT TAA TGT TAT
TGA CTT TTA ACA TGA CAC ATT AAT AAA AAC ACA AAA ATT ACA ATA

        5050        5060        5070        5080
    *     *     *     *     *     *     *     *     *
TTG GTA CTC GAA TTG TAA ATA ACG TCT ACT GCT GTT TAT TCC AGT
AAC CAT GAG CTT AAC ATT TAT TGC AGA TGA CGA CAA ATA AGG TCA

  5090        5100        5110        5120        5130
    *     *     *     *     *     *     *     *     *
TTC TAC TAC CTC AGG TGT CCT ATA GAT TTT TCT TCT ACC AAA GTT
AAG ATG ATG GAG TCC ACA GGA TAT CTA AAA AGA AGA TGG TTT CAA
```

FIGURE 2J

Mouse MEKK1 cDNA

```
         5140          5150          5160          5170
      *     *     *     *     *     *     *     *     *
CAC TCT CAG AAT GAA ATT CTA CGT GCT GTG TGA CTA TGA CTC CTA
GTG AGA GTC TTA CTT TAA GAT GCA CGA CAC ACT GAT ACT GAG GAT

  5180          5190          5200          5210          5220
      *     *     *     *     *     *     *     *     *     *
AGA CTT CCA GGG CTT AAG GGC TAA CTC CTA TTA GCA CCT TAC TAT
TCT GAA GGT CCC GAA TTC CCG ATT GAG GAT AAT CGT GGA ATG ATA

         5230          5240          5250
      *     *     *     *     *     *
GTA AGC AAA TGC TAC AAA AAA AAA AAA AAA AAA
CAT TCG TTT ACG ATG TTT TTT TTT TTT TTT TTT
```

FIGURE 2K

hMEKK1/mMEKK1 Protein Alignment

```
                1          10         20         30         40
mouse   MAAAAGDRAS SSGFPGAAAA SPEAGGGGGG GGALQGSGAP AAGAAGLLRE
human

               50         60         70         80         90
mouse   PGSAGRERAD WRRRQLRKVR SVELDQLPEQ PLFLAAASPP CPSTSPSPEP
human

              100        110        120        130        140
mouse   ADAAAGASRF QPAAGPPPPG AASRCGSHSA ELAAARDSGA RSPAGAEPPS
human

              150        160        170        180        190
mouse   AAAPSGREME NKETLKGLHK MEDRPEERMI REKLKATCMP AWKHEWLERR
human                                                AWKHEWLERR

              200        210        220        230        240
mouse   NRRGPVVVKP IPIKGDGSEV NNLAAEPQGE GQAGSAAAPAP KGRRSPSPGS
human   NRRGPVVVKP IPVKGDGSEM NHLAAESPGE VQASAASPAS KGRRSPSPGN

              250        260        270        280        290
mouse   SPSGRSVKPE SPGVRRKRVS PVPFQSGRIT PPRRAPSPDG FSPYSPEETS
human   SPSGRIVKSE SPGVRRKRVS PVPFQSGRIT PPRRAPSPDG FSPYSPEEIN

              300        310        320        330        340
mouse   RRVNKVMRAR LYLLQQIGPN SFLIGGDSPD NKYRVFIGPQ NCSCGRGARC
human   RRVNKVMRAR LYLLQQIGPN SFLIGGDSPD NKYRVFIGPQ NCSCARGTRC

              350        360        370        380        390
mouse   IHLLFVMLRV FQLEPSDPML WRKTLKNFEV ESLFQKYHSR RSSRIKAPSR
human   IHLLFVMLRV FQLEPSDPML WRKTLKNFEV ESLFQKYHSR RSSRIKAPSR

              400        410        420        430        440
mouse   NTIQKFVSRM SNSHTLSSSS TSTSSSENSI KDEEEQMCPI CLLGMLDEES
human   NTIQKFVSRM SNSHTLSSSS TSTSSSVNSI KDEEEQMCPI CLLGMLDEES
```

FIGURE 3A

hMEKK1/mMEKK1 Protein Alignment

	450	460	470	480	490
mouse	LTVCEDGCRN	KLHHHCMSIW	AEECRRNREP	LICPLCRSKW	RSHDFYSHEL
human	LTVCEDGCRN	KLHHHCMSIW	AEECRRNREP	LICPLCRSKW	RSHDFYSHEL

	500	510	520	530	540
mouse	SSPVESPASL	RAVQQPSSPQ	QPVAGSQRRN	QESSFNLTHF	GTQQIPSAYK
human	SSPVDSPSSL	RAAQQQIVQQ	QPLAGS-RRN	QESNFNLTHY	GTQQIPPAYK

	550	560	570	580	590
mouse	DLAEPWIQVF	GMELVGCLFS	RNWNVREMAL	RRLSHDVSGA	LLLANGESTG
human	DLAEPWIQVF	GMELVGCLFS	RNWNVREMAL	RRLSHDVSGA	LLLANGESTG

	600	610	620	630	640
mouse	NSGGGSGGSL	SAGAASGSSQ	PSISGDVVEA	CCSVLSIVCA	DPVYKVYVAA
human	NSGGGSGGSL	SAGAASGSSQ	PSISGDVVEA	CCSVLSIVCA	DPVYKVYVAA

	650	660	670	680	690
mouse	LKTILRAMLVY	TPCHSLAERI	KLQRLLRPVV	DTILVKCADA	NSRTSQLSIS
human	LKTILRAMLVY	TPCHSLAERI	KLQRLLRPVV	DTILVKCADA	NSRTSQLSIS

	700	710	720	730	740
mouse	TVLELCKGQA	GELAVGREIL	KAGSIGVGGV	DYVLSCILGN	QAESNNWQEL
human	TVLELCNGQA	GKLAVGREIL	KAGSIGVGGV	DYVLSCILGN	QAESNNWQEL

	750	760	770	780	790
mouse	LGRLCLIDRL	LLEFPAEFYP	HIVSIDVSQA	EPVEIRYKKL	LSLLITFALQS
human	LGRLCLIDRL	LLEFPAEFYP	HIVSIDVSQA	EPVEIRYKKL	LSLLITFALQS

	800	810	820	830	840
mouse	IDNSHSMVGK	LSRRIYLSSA	RMVIAVPAVF	SKLVIMLNAS	GSIHFTRMRR
human	IDNSHSMVGK	LSRRIYLSSA	RMVIAVPAVF	SKLVIMLNAS	GSIHFTRMRR

	850	860	870	880	890
mouse	RLMAIADEVE	IAEVIQLGVE	DIVDGHQDSL	QAVAPTSCLE	NSSLEHIVHR
human	RLMAIADEVE	IAEVIQLGVE	DIVDGHQDSL	QALAPASCLE	NSSLEHIVHR

FIGURE 3B

hMEKK1/mMEKK1 Protein Alignment

	900	910	920	930	940
mouse	EKTGKGLSAT	RLSASSEDIS	DRLAGVSVGL	PSSTTTEQPK	PAVQTKGRPH
human	EKTGKGLSAT	RLSASSEDIS	DRLAGVSVGL	PSSTTTEQPK	PAVQTKGRPH
	950	960	970	980	990
mouse	SQCLNSSPLS	HAQLMFPAPS	APCSSAPSVP	DISKHRPQAF	VPCKIPSASP
human	SQCLNSSPLS	HAQLMFPAPS	APCSSAPSVP	DISKHRPQAF	VPCKIPSASP
	1000	1010	1020	1030	1040
mouse	QTQRKFSLQF	QRNCSEHRDS	DQLSPVFTQS	RPPPSSNIHR	PKPSRPVPGS
human	QTQRKFSLQF	QRNCSEHRDS	DQLSPVFTQS	RPPPSSNIHR	PKPSRPVPGS
	1050	1060	1070	1080	1090
mouse	TSKLGDATKS	SMTLDLGSAS	RCDDSFGGGG	NSGNAVIPSD	EIVFTPVEDK
human	TSKLGDATKS	SMTLDLGSAS	RCDDSFGGGG	NSGNAVIPSD	EIVFTPVEDK
	1100	1110	1120	1130	1140
mouse	CRLDVNTELN	SSIEDLLEAS	MPSSDITVIF	KSEVAVLSPE	KAENDDTYKD
human	CRLDVNTELN	SSIEDLLEAS	MPSSDITVIF	KSEVAVLSPE	KAENDDTYKD
	1150	1160	1170	1180	1190
mouse	DVNHNQKCKE	KMEAEEEEAL	AIAMAMSASQ	DALPIVPQLQ	VENGEDIIII
human	DVNHNQKCKE	KMEAEEEEAL	AIAMAMSASQ	DALPIVPQLQ	VENGEDIIII
	1200	1210	1220	1230	1240
mouse	QQDTPETLPG	HIKAKQPYRE	DAEWLKGQQI	GLGAFSSCYQ	AQDVGIGTLM
human	QQDTPETLPG	HIKAKQPYRE	DAEWLKGQQI	GLGAFSSCYQ	AQDVGIGTLM
	1250	1260	1270	1280	1290
mouse	AVKQVTYVRN	TSSEQEEVVE	ALREEIRMMG	HLNHPNIIRM	LGATCEKSNY
human	AVKQVTYVRN	TSSEQEEVVE	ALREEIRMMS	HLNHPNIIRM	LGATCEKSNY
	1300	1310	1320	1330	1340
mouse	NLFIEWMAGG	SVAHLLSKYG	AFKESVVINY	TEQLLRGLSY	LHENQIIHRD
human	NLFIEWMAGG	SVAHLLSKYG	AFKESVVINY	TEQLLRGLSY	LHENQIIHRD

FIGURE 3C

hMEKK1/mMEKK1 Protein Alignment

	1350	1360	1370	1380	1390
mouse	VKGANLLIDS	TGQRLRIADF	GAAARLASKG	TGAGEFQGQL	LGTIAFMAPE
human	VKGANLLIDS	TGQRLRIADF	GAAARLASKG	TGAGEFQGQL	LGTIAFMAPE

	1400	1410	1420	1430	1440
mouse	VLRGQQYGRS	CDVWSVGCAI	IEMACAKPPW	NAEKHSNHLA	LIFKIASATT
human	VLRGQQYGRS	CDVWSVGCAI	IEMACAKPPW	NAEKHSNHLA	LIFKIASATT

	1450	1460	1470	1480	1490
mouse	APSIPSHLSP	GLRDVAVRCL	ELQPQDRPPS	RELLKHPVFR	TIW*
human	APSIPSHLSP	GLRDVALRCL	ELQPQDRPPS	RELLKHPVFR	TIW*

FIGURE 3D

FIG.4

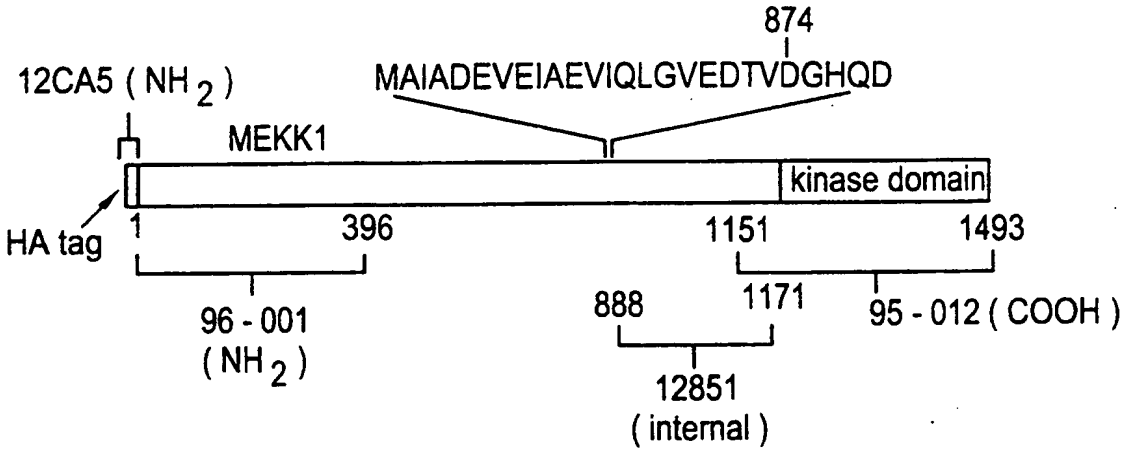

874
12CA5 (NH 2)
MAIADEVEIAEVIQLGVEDTVDGHQD
MEKK1
kinase domain
HA tag
1
396
1151
1493
96 - 001
(NH 2)
888
1171
95 - 012 (COOH)
12851
(internal)

FIG.5

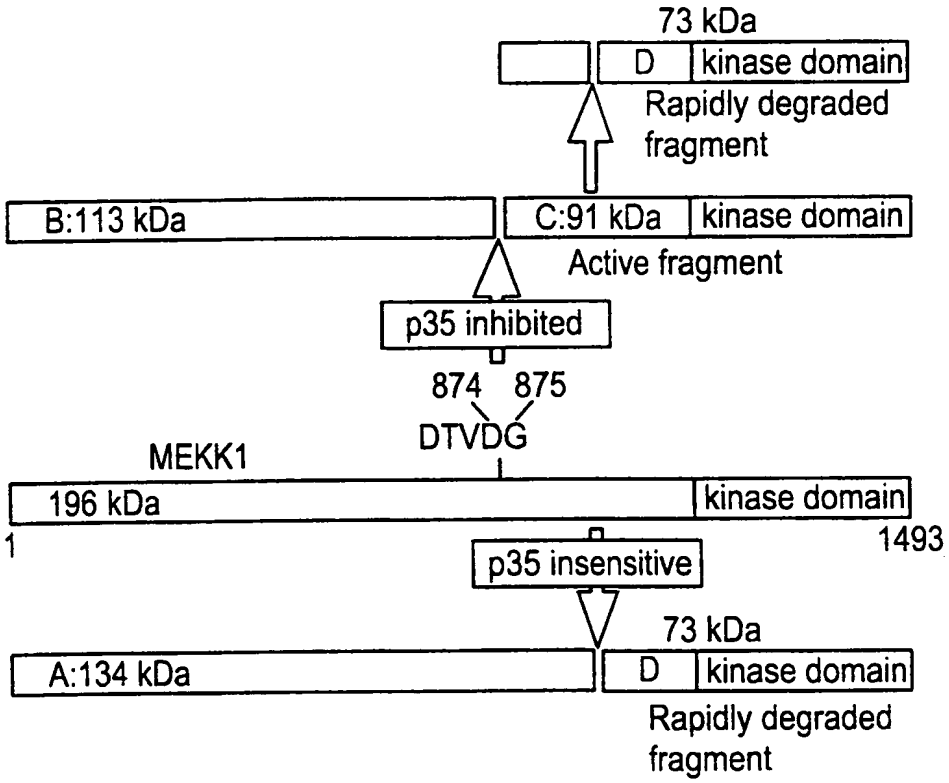
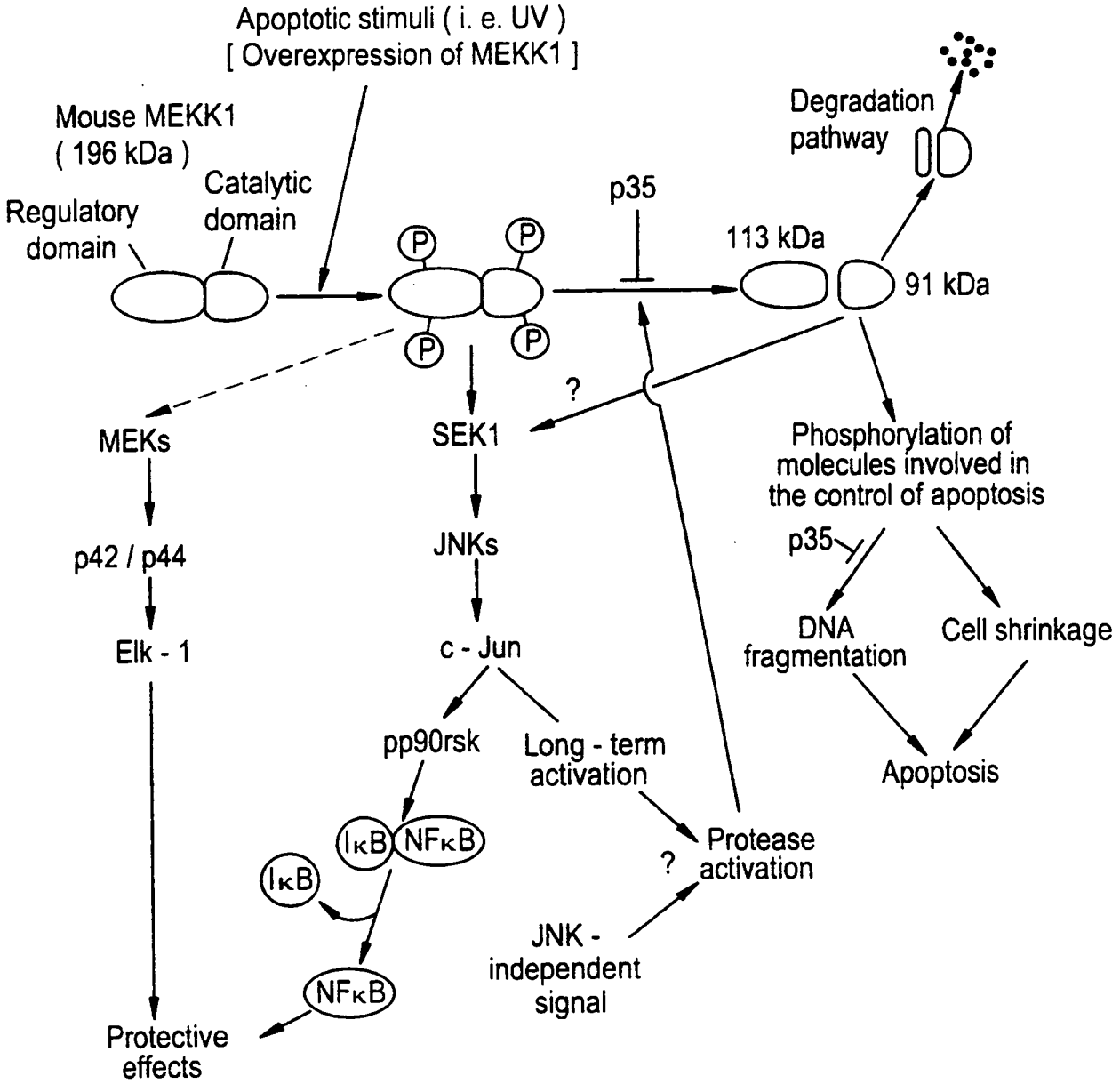

73 kDa
D
kinase domain
Rapidly degraded fragment
B:113 kDa
C:91 kDa
kinase domain
Active fragment
p35 inhibited
874
875
DTVDG
MEKK1
196 kDa
kinase domain
1
1493
p35 insensitive
73 kDa
A:134 kDa
D
kinase domain
Rapidly degraded fragment

FIG.6


Apoptotic stimuli (i. e. UV)
[Overexpression of MEKK1]
Mouse MEKK1
(196 kDa)
Regulatory domain
Catalytic domain
P
p35
113 kDa
91 kDa
Degradation pathway
?
MEKs
SEK1
Phosphorylation of molecules involved in the control of apoptosis
p42 / p44
JNKs
p35
DNA fragmentation
Cell shrinkage
Elk - 1
c - Jun
pp90rsk
Long - term activation
Apoptosis
IκB
NFκB
Protease activation
JNK - independent signal
Protective effects

Lipman-Pearson Protein Alignment
Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

		Similarity Index	Gap Number	Gap Length	Consensus Length
(1>1493)	(1>1493)	94.1	4	10	1498

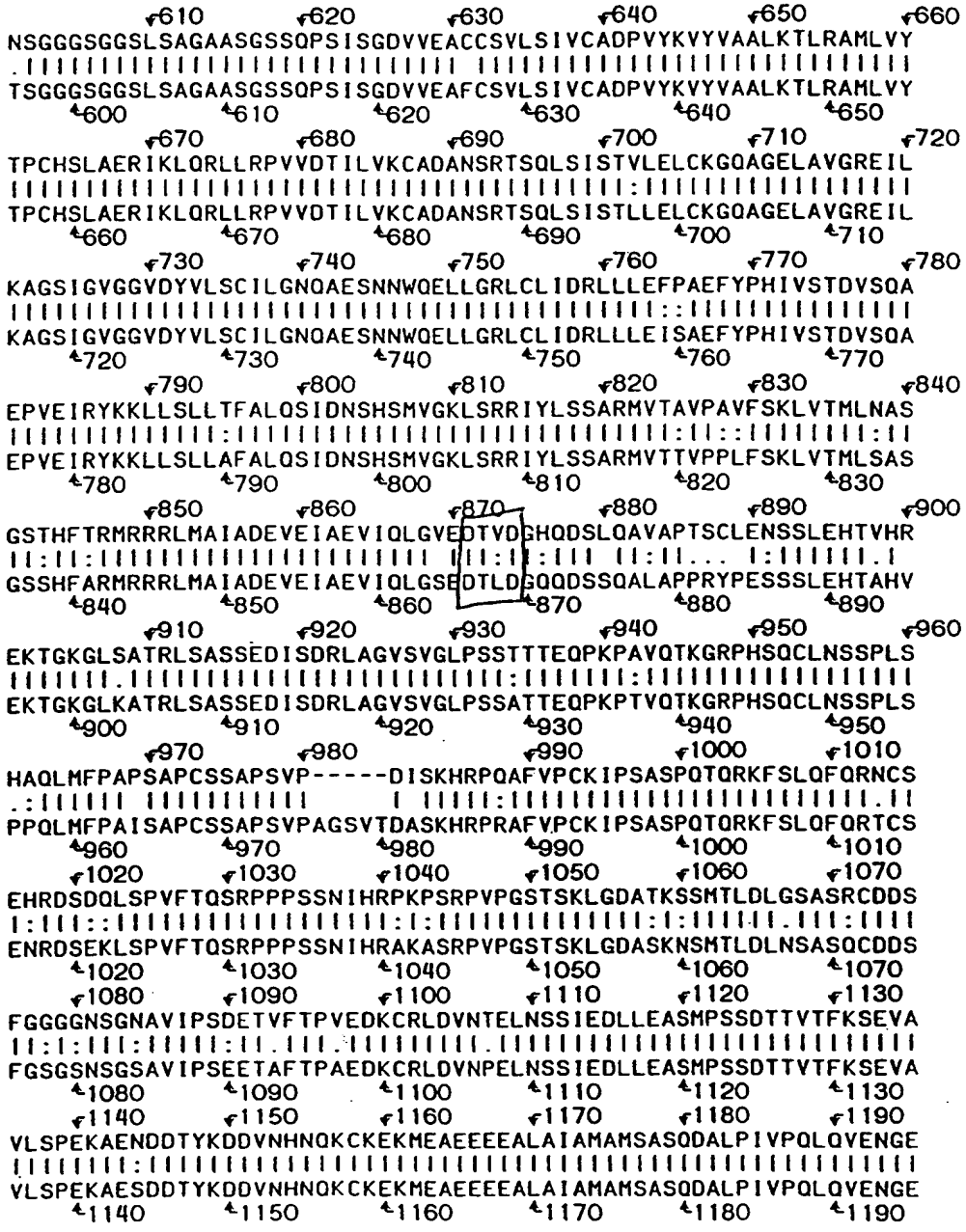


FIGURE 7A

Lipman-Pearson Protein Alignment
Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

		Similarity Index	Gap Number	Gap Length	Consensus Length
(1>1493)	(1>1493)	94.1	4	10	1498

```
          10        20        30        40        50        60
MAAAAGDRASSSGFPGAAAASPEAGGGGGGGGALQGSGAPAAGAAGLLREPGSAGRERAD
||||||||||||||||||||||||  ||||||||||||||||  |||||.|||||||||
MAAAAGDRASSSGFPGAAAASPEA---GGGGGALQGSGAPAAGA-GLLRETGSAGRERAD
          10        20        30        40        50

          70        80        90       100       110       120
WRRRQLRKVRSVELDQLPEQPLFLAAASPPCPSTSPSPEPADAAAGASRFQPAAGPPPPG
|||:|||||||||||||||||||||:| ||||||||||||||||||| ||||||||||
WRRQQLRKVRSVELDQLPEQPLFLTA-SPPCPSTSPSPEPADAAAGASGFQPAAGPPPPG
    60        70        80        90       100       110

         130       140       150       160       170       180
AASRCGSHSAELAAARDSGARSPAGAEPPSAAAPSGREMENKETLKGLHKMEDRPEERMI
||||||||||||||||||||||||||||||||||||||||||||||||||:||||||||
AASRCGSHSAELAAARDSGARSPAGAEPPSAAAPSGREMENKETLKGLHKMDDRPEERMI
    120       130       140       150       160       170

         190       200       210       220       230       240
REKLKATCMPAWKHEWLERRNRRGPVVVKPIPIKGDGSEVNNLAAEPQGEGQAGSAAPAP
||||||||||||||||||||||||||||||||||||||::||||| ||||||||||||
REKLKATCMPAWKHEWLERRNRRGPVVVKPIPIKGDGSEMSNLAAELQGEGQAGSAAPAP
    180       190       200       210       220       230

         250       260       270       280       290       300
KGRRSPSPGSSPSGRSVKPESPGVRRKRVSPVPFQSGRITPPRRAPSPDGFSPYSPEETS
|||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
KGRRSPSPGSSPSGRSGKPESPGVRRKRVSPVPFQSGRITPPRRAPSPDGFSPYSPEETS
    240       250       260       270       280       290

         310       320       330       340       350       360
RRVNKVMRARLYLLQQIGPNSFLIGGDSPDNKYRVFIGPQNCSCGRGAFCIHLLFVMLRV
||||||||||||||||||||||||||||||||||||||||||||||:||||||||||||
RRVNKVMRARLYLLQQIGPNSFLIGGDSPDNKYRVFIGPQNCSCGRGTFCIHLLFVMLRV
    300       310       320       330       340       350

         370       380       390       400       410       420
FQLEPSDPMLWRKTLKNFEVESLFQKYHSRRSSRIKAPSRNTIQKFVSRMSNSHTLSSSS
||||||||||||||||||||||||||||||||||||||||||||||||.|||||||||
FQLEPSDPMLWRKTLKNFEVESLFQKYHSRRSSRIKAPSRNTIQKFVSRMSNCHTLSSSS
    360       370       380       390       400       410

         430       440       450       460       470       480
TSTSSSENSIKDEEEQMCPICLLGMLDEESLTVCEDGCRNKLHHHCMSIWAEECRRNREP
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TSTSSSENSIKDEEEQMCPICLLGMLDEESLTVCEDGCRNKLHHHCMSIWAEECRRNREP
    420       430       440       450       460       470

         490       500       510       520       530       540
LICPLCRSKWRSHDFYSHELSSPVESPASLRAVQQPSSPQQPVAGSQRRNQESSFNLTHF
|||||||||||||||||||||||:||:|||:|||||||||||||||||||:|||||:
LICPLCRSKWRSHDFYSHELSSPVDSPTSLRGVQQPSSPQQPVAGSQRRNQESNFNLTHY
    480       490       500       510       520       530

         550       560       570       580       590       600
GTQQIPSAYKDLAEPWIQVFGMELVGCLFSRNWNVREMALRRLSHDVSGALLLANGESTG
||||||:||||||||||.||||||||||||||||||||||||||||||||||||||||||
GTQQIPPAYKDLAEPWIQAFGMELVGCLFSRNWNVREMALRRLSHDVSGALLLANGESTG
    540       550       560       570       580       590
```

FIGURE 7B

Lipman-Pearson Protein Alignment
Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

		Similarity Index	Gap Number	Gap Length	Consensus Length
(1>1493)	(1>1493)	94.1	4	10	1498

```
     1200      1210      1220      1230      1240      1250
DIIIIQQDTPETLPGHTKAKQPYREDAEWLKGQQIGLGAFSSCYQAQDVGTGTLMAVKQV
||||||||||||||||||||::|||||:||||||||||||||||||||||||||||||
DIIIIQQDTPETLPGHTKANEPYREDTEWLKGQQIGLGAFSSCYQAQDVGTGTLMAVKQV
     1200      1210      1220      1230      1240      1250
     1260      1270      1280      1290      1300      1310
TYVRNTSSEQEEVVEALREEIRMMGHLNHPNIIRMLGATCEKSNYNLFIEWMAGGSVAHL
||||||||||||||||||||||||:|||||||||||||||||||||||||||:|||||
TYVRNTSSEQEEVVEALREEIRMMSHLNHPNIIRMLGATCEKSNYNLFIEWMAGASVAHL
     1260      1270      1280      1290      1300      1310
     1320      1330      1340      1350      1360      1370
LSKYGAFKESVVINYTEQLLRGLSYLHENQIIHRDVKGANLLIDSTGQRLRIADFGAAAR
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
LSKYGAFKESVVINYTEQLLRGLSYLHENQIIHRDVKGANLLIDSTGQRLRIADFGAAAR
     1320      1330      1340      1350      1360      1370
     1380      1390      1400      1410      1420      1430
LASKGTGAGEFQGQLLGTIAFMAPEVLRGQQYGRSCDVWSVGCAIIEMACAKPPWNAEKH
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
LASKGTGAGEFQGQLLGTIAFMAPEVLRGQQYGRSCDVWSVGCAIIEMACAKPPWNAEKH
     1380      1390      1400      1410      1420      1430
     1440      1450      1460      1470      1480      1490
SNHLALIFKIASATTAPSIPSHLSPGLRDVAVRCLELQPQDRPPSRELLKHPVFRTTW
||||||||||||||||||||||||||||:||||||||||||||||||||||||||||
SNHLALIFKIASATTAPSIPSHLSPGLRDVALRCLELQPQDRPPSRELLKHPVFRTTW
     1440      1450      1460      1470      1480      1490
```

FIGURE 7C

MEKK 1

```
rat      MAAAAGDRASSSGFPGAAAASPEA---GGGGGALQGSGAPAAG-AGLLRETGSAGRE
mouse    MAAAAGDRASSSGFPGAAAASPEAGGGGGGGGGALQGSGAPAAGAAGLLREPGSAGRE

rat      RADWRRQQLRKVRSVELDQLPEQPLFL-TASPPCPSTSPSPEPADAAAGASGFQPAA
mouse    RADWRRRQLRKVRSVELDQLPEQPLFLAAASPPCPSTSPSPEPADAAAGASRFQPAA

rat      GPPPPGAASRCGSHSAELAAARDSGARSPAGAEPPSAAAPSGREMENKETLKGLHKM
mouse    GPPPPGAASRCGSHSAELAAARDSGARSPAGAEPPSAAAPSGREMENKETLKGLHKM

rat      DDRPEERMIREKLKATCMPAWKHEWLERRNRRGPVVVKPIPIKGDGSEMSNLAAELQ
mouse    EDRPEERMIREKLKATCMPAWKHEWLERRNRRGPVVVKPIPIKGDGSEVNNLAAEPQ

rat      GEGQAGSAAPAPKGRRSPSPGSSPSGRSGKPESPGVRRKRVSPVPFQSGRITPPRRA
mouse    GEGQAGSAAPAPKGRRSPSPGSSPSGRSVKPESPGVRRKRVSPVPFQSGRITPPRRA

rat      PSPDGFSPYSPEETSRRVNKVMRARLYLLQQIGPNSFLIGGDSPDNKYRVFIGPQNC
mouse    PSPDGFSPYSPEETSRRVNKVMRARLYLLQQIGPNSFLIGGDSPDNKYRVFIGPQNC

rat      SCGRGTFCIHLLFVMLRVFQLEPSDPMLWRKTLKNFEVESLFQKYHSRRSSRIKAPS
mouse    SCGRGAFCIHLLFVMLRVFQLEPSDPMLWRKTLKNFEVESLFQKYHSRRSSRIKAPS

rat      RNTIQKFVSRMSNCHTLSSSSTSTSSSENSIKDEEEQMCPICLLGMLDEESLTVCED
mouse    RNTIQKFVSRMSNSHTLSSSSTSTSSSENSIKDEEEQMCPICLLGMLDEESLTVCED

rat      GCRNKLHHHCMSIWAEECRRNREPLICPLCRSKWRSHDFYSHELSSPVDSPTSLRGV
mouse    GCRNKLHHHCMSIWAEECRRNREPLICPLCRSKWRSHDFYSHELSSPVESPASLRAV
human       NKLHHHCMSIWAEECRRN  PLICPLCRS WRSHDFYSHELSSPVDSPSSL

rat      QQPSSPQQPVAGSQRRNQESNFNLTHYGTQQIPPAYKDLAEPWIQAFGMELVGCLFS
mouse    QQPSSPQQPVAGSQRRNQESSFNLTHFGTQQIPSAYKDLAEPWIQVFGMELVGCLFS
human     Q  V  HPLAGS RRNQESNFNLTHYGTQQIPPAYKDLAEPWIQVFGMELVGCLFS

rat      RNWNVREMALRRLSHDVSGALLLANGESTGTSGGGSGGSLSAGAASGSSQPSISGDV
mouse    RNWNVREMALRRLSHDVSGALLLANGESTGNSGGGSGGSLSAGAASGSSQPSISGDV
human    RNWNVREMALRRLSHDVSGALLLANGESTGNSGGSSGSSPSGGATSG SQTS SGDV

rat      VEAFCSVLSIVCADPVYKVYVAALKTLRAMLVYTPCHSLAERIKLQRLLRPVVDTIL
mouse    VEACCSVLSIVCADPVYKVYVAALKTLRAMLVYTPCHSLAERIKLQRLLRPVVDTIL
human    VEACC

rat      VKCADANSRTSQLSISTVLELCKGQAGELAVGREILKAGSIGVGGVDYVLSCILGNQ
mouse    VKCADANSRTSQLSISTVLELCKGQAGELAVGREILKAGSIGVGGVDYVLSCILGNQ
human

rat      AESNNWQELLGRLCLIDRLLLEISAEFYPHIVSTDVSQAEPVEIRYKKLLSLLAFAL
mouse    AESNNWQELLGRLCLIDRLLLEFPAEFYPHIVSTDVSQAEPVEIRYKKLLSLLTFAL
human                                PAEFYPHIVSTDVSQAEPVEIRYKKLLSLL FA

rat      QSIDNSHSMVGKLSRRIYLSSARMVTTVPPIFSKLVTMLSASGSSHFARMRRRLMAI
mouse    QSIDNSHSMVGKLSRRIYLSSARMVTAVPAVFSKLVTMLNASGSTHFTRMRRRLMTI
human    K ID SHSMVG  SR DIS LCYDDGRSAVCFPSW PCLMLLGSTHFTRMRRRLMAI
```

FIGURE 8A

```
rat      ADEVEIAEVIQLGSEDTLDGQQDSSQALAPPRYPESSSLEHTAHVEKTGKGLKATRL
mouse    ADEVEIAEVIQLGVEDTVDGHQDSLQAVAPTSCLENSSLEHTVHREKTGKGLSATRL
human    ADEVEIAEVIQLGEVDTVDGHQDSLRALAPASCRENSSLEHTVHREKTGKGLSATRL

rat      SASSEDISDRLAGVSVGLPSSATTEQPKPTVQTKGRPHSQCLNSSPLSPPQLMFPAI
mouse    SASSEDISDRLAGVSVGLPSSTTTEQPKPAVQTKGRPHSQCLNSSPLSHAQLMFPAP
human    STSSEEISDRLAGVSVGFPSSTTTEQPKPAVQTKGRPHSQCLNSSPLSHAQLMFPAP

rat      SAPCSSAPSVPAGSVTDASKHRPRAFVPCKIPSASPQTQRKFSLQFQRTCSENRDSE
mouse    SAPCSSAPSVP-----DISKHRPQAFVPCKIPSASPQTQRKFSLQFQRNCSEHRDSD
human    SAPCSSAP VP     DISKHRPQAFVPCKILPHLPQTQRKFSLQFQRN  EHRDQT

rat      KLSPVFTQSRPPPSSNIHRAKASRPVPGSTSKLGDASKNSMTLDLNSASQCDDSFGS
mouse    QLSPVFTQSRPPPSSNIHRPKPSRPVPGSTSKLGDATKSSMTLDLGSASRCDDSFGG
human    QLSPVFTQSQDPTSSNIHRPKPDRPAPGSTSKLGDATKSSMTLDLGQCSRCDDSFGG

rat      GSNSGSAVIPSEETAFTPAEDKCRLDVNPELNSSIEDLLEASMPSSDTTVTFKSEVA
mouse    GGNSGNAVIPSDETVFTPVEDKCRLDVNTELNSSIEDLLEASMPSSDTTVTFKSEVA
human    GGNSGNAVIPSDETVFTPVEDKCRLDVNTELNSSIEDLLEASMPSSDTTVTFKSEVA

rat      VLSPEKAESDDTYKDDVNHNQKCKEKMEAEEEEALAIAMAMSASQDALPIVPQLQVE
mouse    VLSPEKAENDDTYKDDVNHNQKCKEKMEAEEEEALAIAMAMSASQDALPIVPQLQVE
human    VLSPEKAENDDTYK  VY

rat      NGEDIIIIQQDTPETLPGHTKANEPYREDTEWLKGQQIGLGAFSSCYQAQDVGTGTL
mouse    NGEDIIIIQQDTPETLPGHTKAKQPYREDAEWLKGQQIGLGAFSSCYQAQDVGTGTL
human          VIQQDTPETLPGHTKAKQPYREDAEWL G QIGLGHF

rat      MAVKQVTYVRNTSSEQEEVVEALREEIRMMSHLNHPNIIRMLGATCEKSNYNLFIEW
mouse    MAVKQVTYVRNTSSEQEEVVEALREEIRMMGHLNHPNIIRMLGATCEKSNYNLFIEW
human                                EEIR MSHLNHP IIRMLG TGKKSNY LFIEW

rat      MAGASVAHLLSKYGAFKESVVINYTEQLLRGLSYLHENQIIHRDVKGANLLIDSTGQ
mouse    MAGGSVAHLLSKYGAFKESVVINYTEQLLRGLSYLHENQIIHRDVKGANLLIDSTGQ
human    MAGGSVAHLLSKYGAF ESVVI YTEQ LRGLSYLHENQIIH DVKGANLLID TG

rat      RLRIADFGAAARLASKGTGAGEFQGQLLGTIAFMAPEVLRGQQYGRSCDVWSVGCAI
mouse    RLRIADFGAAARLASKGTGAGEFQGQLLGTIAFMAPEVLRGQQYGRSCDVWSVGCAI
human    RLRIADFGAAA LASKG GAGEFQGQL GTIAFMAPEV RG QYGRSCDVWSVGCAI

rat      IEMACAKPPWNAEKHSNHLALIFKIASATTAPSIPSHLSPGLRDVALRCLELQPQDR
mouse    IEMACAKPPWNAEKHSNHLALIFKIASATTAPSIPSHLSPGLRDVAVRCLELQPQDR
human    IEMACAKPPWNAEKHSNHLALIKKIASATTAPSIPSHLSPGLRNVALRCLELQPQDR

rat      PPSRELLKHPVFRTTW
mouse    PPSRELLKHPVFRTTW
human    PPSRELLKHPVFRTT
```

FIGURE 8B

SEQUENCE LISTING

<110> Johnson, Gary L.

<120> MEKK1 PROTEINS AND FRAGMENTS THEREOF FOR USE IN REGULATING APOPTOSIS

<130> CPI-042CPPC

<140> PCT/US99/02974
<141> February 12, 1999

<160> 21

<170> PatentIn Ver. 2.0

<210> 1
<211> 3260
<212> DNA
<213> Murine

<221> CDS
<222> (486)..(2501)

<400> 1
tacactcctt gccacagtct ggcagaaaga atcaaacttc agagactcct ccggccagtt 60

gtagacacta tccttgtcaa gtgtgcagat ccaacagccg cacgagtcag ctgtccatat 120

ctacagtgct ggaactctgc aagggccaag caggagagct ggcggttggg agagaaatac 180

ttaaagctgg gtccatcggg gttggtggtg tcgattacgt cttaagttgt atccttggaa 240

accaagctga atcaaacaac tggcaagaac tgctgggtcg cctctgtctt atagacaggt 300

tgctgttgga atttcctgct gaattctatc ctcatattgt cagtactgat gtctcacaag 360

ctgagcctgt tgaaatcagg tacaagaagc tgctctccct cttaaccttt gccttgcaat 420

ccattgacaa ttcccactcg atggttggca agctctctcg gaggatatat ctgagctctg 480

ccagg atg gtg acc gca gtg ccc gct gtg ttt tcc aag ctg gta acc atg 530
Met Val Thr Ala Val Pro Ala Val Phe Ser Lys Leu Val Thr Met
1 5 10 15

ctt aat gct tct ggc tcc acc cac ttc acc agg atg cgc cgg cgt ctg 578
Leu Asn Ala Ser Gly Ser Thr His Phe Thr Arg Met Arg Arg Arg Leu
20 25 30

atg gct atc gcg gat gag gta gaa att gcc gag gtc atc cag ctg ggt 626
Met Ala Ile Ala Asp Glu Val Glu Ile Ala Glu Val Ile Gln Leu Gly
35 40 45

gtg gag gac act gtg gat ggg cat cag gac agc tta cag gcc gtg gcc 674
Val Glu Asp Thr Val Asp Gly His Gln Asp Ser Leu Gln Ala Val Ala
50 55 60

ccc acc agc tgt cta gaa aac agc tcc ctt gag cac aca gtc cat aga 722
Pro Thr Ser Cys Leu Glu Asn Ser Ser Leu Glu His Thr Val His Arg
65 70 75

gag aaa act gga aaa gga cta agt gct acg aga ctg agt gcc agc tcg 770
Glu Lys Thr Gly Lys Gly Leu Ser Ala Thr Arg Leu Ser Ala Ser Ser

```
80                  85                  90                  95

gag gac att tct gac aga ctg gcc ggc gtc tct gta gga ctt ccc agc   818
Glu Asp Ile Ser Asp Arg Leu Ala Gly Val Ser Val Gly Leu Pro Ser
                100                 105                 110

tca aca aca aca gaa caa cca aag cca gcg gtt caa aca aaa ggc aga   866
Ser Thr Thr Thr Glu Gln Pro Lys Pro Ala Val Gln Thr Lys Gly Arg
            115                 120                 125

ccc cac agt cag tgt ttg aac tcc tcc cct ttg tct cat gct caa tta   914
Pro His Ser Gln Cys Leu Asn Ser Ser Pro Leu Ser His Ala Gln Leu
        130                 135                 140

atg ttc cca gca cca tca gcc cct tgt tcc tct gcc ccg tct gtc cca   962
Met Phe Pro Ala Pro Ser Ala Pro Cys Ser Ser Ala Pro Ser Val Pro
    145                 150                 155

gat att tct aag cac aga ccc cag gca ttt gtt ccc tgc aaa ata cct  1010
Asp Ile Ser Lys His Arg Pro Gln Ala Phe Val Pro Cys Lys Ile Pro
160                 165                 170                 175

tcc gca tct cct cag aca cag cgc aag ttc tct cta caa ttc cag agg  1058
Ser Ala Ser Pro Gln Thr Gln Arg Lys Phe Ser Leu Gln Phe Gln Arg
                180                 185                 190

aac tgc tct gaa cac cga gac tca gac cag ctc tcc cca gtc ttc act  1106
Asn Cys Ser Glu His Arg Asp Ser Asp Gln Leu Ser Pro Val Phe Thr
            195                 200                 205

cag tca aga ccc cca ccc tcc agt aac ata cac agg cca aag cca tcc  1154
Gln Ser Arg Pro Pro Pro Ser Ser Asn Ile His Arg Pro Lys Pro Ser
        210                 215                 220

cga ccc gtt ccg ggc agt aca agc aaa cta ggg gac gcc aca aaa agt  1202
Arg Pro Val Pro Gly Ser Thr Ser Lys Leu Gly Asp Ala Thr Lys Ser
    225                 230                 235

agc atg aca ctt gat ctg ggc agt gct tcc agg tgt gac gac agc ttt  1250
Ser Met Thr Leu Asp Leu Gly Ser Ala Ser Arg Cys Asp Asp Ser Phe
240                 245                 250                 255

ggc ggc ggc ggc aac agt ggc aac gcc gtc ata ccc agc gac gag aca  1298
Gly Gly Gly Gly Asn Ser Gly Asn Ala Val Ile Pro Ser Asp Glu Thr
                260                 265                 270

gtg ttc acg ccg gtg gag gac aag tgc agg tta gat gtg aac acc gag  1346
Val Phe Thr Pro Val Glu Asp Lys Cys Arg Leu Asp Val Asn Thr Glu
            275                 280                 285

ctc aac tcc agc atc gag gac ctt ctt gaa gca tcc atg cct tca agt  1394
Leu Asn Ser Ser Ile Glu Asp Leu Leu Glu Ala Ser Met Pro Ser Ser
        290                 295                 300

gac acg aca gtc act ttc aag tcc gaa gtc gcc gtc ctc tct ccg gaa  1442
Asp Thr Thr Val Thr Phe Lys Ser Glu Val Ala Val Leu Ser Pro Glu
    305                 310                 315

aag gcc gaa aat gac gac acc tac aaa gac gac gtc aat cat aat caa  1490
Lys Ala Glu Asn Asp Asp Thr Tyr Lys Asp Asp Val Asn His Asn Gln
320                 325                 330                 335
```

```
aag tgc aaa gaa aag atg gaa gct gaa gag gag gag gct tta gcg atc   1538
Lys Cys Lys Glu Lys Met Glu Ala Glu Glu Glu Glu Ala Leu Ala Ile
                340                 345                 350

gcc atg gcg atg tca gcg tct cag gat gcc ctc ccc atc gtc cct cag   1586
Ala Met Ala Met Ser Ala Ser Gln Asp Ala Leu Pro Ile Val Pro Gln
            355                 360                 365

ctg cag gtg gaa aat gga gaa gat att atc atc att cag cag gac aca   1634
Leu Gln Val Glu Asn Gly Glu Asp Ile Ile Ile Ile Gln Gln Asp Thr
        370                 375                 380

cca gaa act ctt cca gga cat acc aaa gcg aaa cag cct tac aga gaa   1682
Pro Glu Thr Leu Pro Gly His Thr Lys Ala Lys Gln Pro Tyr Arg Glu
    385                 390                 395

gac gct gag tgg ctg aaa ggc cag cag ata ggc ctc gga gca ttt tct   1730
Asp Ala Glu Trp Leu Lys Gly Gln Gln Ile Gly Leu Gly Ala Phe Ser
400                 405                 410                 415

tcc tgt tac caa gca cag gat gtg ggg act ggg act tta atg gct gtg   1778
Ser Cys Tyr Gln Ala Gln Asp Val Gly Thr Gly Thr Leu Met Ala Val
                420                 425                 430

aaa cag gtg acg tac gtc aga aac aca tcc tcc gag cag gag gag gtg   1826
Lys Gln Val Thr Tyr Val Arg Asn Thr Ser Ser Glu Gln Glu Glu Val
            435                 440                 445

gtg gaa gcg ttg agg gaa gag atc cgg atg atg ggt cac ctc aac cat   1874
Val Glu Ala Leu Arg Glu Glu Ile Arg Met Met Gly His Leu Asn His
        450                 455                 460

cca aac atc atc cgg atg ctg ggg gcc acg tgc gag aag agc aac tac   1922
Pro Asn Ile Ile Arg Met Leu Gly Ala Thr Cys Glu Lys Ser Asn Tyr
    465                 470                 475

aac ctc ttc att gag tgg atg gcg gga gga tct gtg gct cac ctc ttg   1970
Asn Leu Phe Ile Glu Trp Met Ala Gly Gly Ser Val Ala His Leu Leu
480                 485                 490                 495

agt aaa tac gga gct ttc aag gag tca gtc gtc att aac tac act gag   2018
Ser Lys Tyr Gly Ala Phe Lys Glu Ser Val Val Ile Asn Tyr Thr Glu
                500                 505                 510

cag tta ctg cgt ggc ctt tcc tat ctc cac gag aac cag atc att cac   2066
Gln Leu Leu Arg Gly Leu Ser Tyr Leu His Glu Asn Gln Ile Ile His
            515                 520                 525

aga gac gtc aaa ggt gcc aac ctg ctc att gac agc acc ggt cag agg   2114
Arg Asp Val Lys Gly Ala Asn Leu Leu Ile Asp Ser Thr Gly Gln Arg
        530                 535                 540

ctg aga att gca gac ttt gga gct gct gcc agg ttg gca tca aaa gga   2162
Leu Arg Ile Ala Asp Phe Gly Ala Ala Ala Arg Leu Ala Ser Lys Gly
    545                 550                 555

acc ggt gca gga gag ttc cag gga cag tta ctg ggg aca att gca ttc   2210
Thr Gly Ala Gly Glu Phe Gln Gly Gln Leu Leu Gly Thr Ile Ala Phe
560                 565                 570                 575
```

```
atg gcg cct gag gtc cta aga ggt cag cag tat ggt agg agc tgt gat   2258
Met Ala Pro Glu Val Leu Arg Gly Gln Gln Tyr Gly Arg Ser Cys Asp
                580                 585                 590

gta tgg agt gtt ggc tgc gcc att ata gaa atg gct tgt gca aaa cca   2306
Val Trp Ser Val Gly Cys Ala Ile Ile Glu Met Ala Cys Ala Lys Pro
            595                 600                 605

cct tgg aat gca gaa aaa cac tcc aat cat ctc gcc ttg ata ttt aag   2354
Pro Trp Asn Ala Glu Lys His Ser Asn His Leu Ala Leu Ile Phe Lys
        610                 615                 620

att gct agc gca act act gca ccg tcc atc ccg tca cac ctg tcc ccg   2402
Ile Ala Ser Ala Thr Thr Ala Pro Ser Ile Pro Ser His Leu Ser Pro
    625                 630                 635

ggt ctg cgc gac gtg gcc gtg cgc tgc tta gaa ctt cag cct cag gac   2450
Gly Leu Arg Asp Val Ala Val Arg Cys Leu Glu Leu Gln Pro Gln Asp
640                 645                 650                 655

cgg cct ccg tcc aga gag ctg ctg aaa cat ccg gtc ttc cgt acc acg   2498
Arg Pro Pro Ser Arg Glu Leu Leu Lys His Pro Val Phe Arg Thr Thr
                660                 665                 670

tgg tagttaattg ttcagatcag ctctaatgga gacaggatat cgaaccggga       2551
Trp

gagagaaaag agaacttgtg ggcgaccatg ccgctaaccg cagccctcac gccactgaac 2611

agccagaaac ggggccagcg gggaaccgta cctaagcatg tgattgacaa atcatgacct 2671

gtacctaagc tcgatatgca gacatctaca gctcgtgcag gaactgcaca ccgtgccttt 2731

cacaggactg gctctggggg accaggaagg cgatggagtt tgcatgacta aagaacagaa 2791

gcataaattt atttttggag cacttttca gctaatcagt attaccatgt acatcaacat 2851

gcccgccaca tttcaaactc agactgtccc agatgtcaag atccactgtg tttgagtttg 2911

tttgcagttc cctcagcttg ctggtaattg tggtgttttg ttttcgatgc aaatgtgatg 2971

taatattctt attttctttg gatcaaagct ggactgaaaa ttgtactgtg taattatttt 3031

tgtgttttta atgttatttg gtactcgaat tgtaaataac gtctactgct gtttattcca 3091

gtttctacta cctcaggtgt cctatagatt tttcttctac caaagttcac tctcagaatg 3151

aaattctacg tgctgtgtga ctatgactcc taagacttcc agggcttaag ggctaactcc 3211

tattagcacc ttactatgta agcaaatgct acaaaaaaaa aaaaaaaaa             3260
```

<210> 2
<211> 672
<212> PRT
<213> Murine

```
<400> 2
Met Val Thr Ala Val Pro Ala Val Phe Ser Lys Leu Val Thr Met Leu
  1               5                  10                  15
```

Asn Ala Ser Gly Ser Thr His Phe Thr Arg Met Arg Arg Arg Leu Met
20 25 30

Ala Ile Ala Asp Glu Val Glu Ile Ala Glu Val Ile Gln Leu Gly Val
35 40 45

Glu Asp Thr Val Asp Gly His Gln Asp Ser Leu Gln Ala Val Ala Pro
50 55 60

Thr Ser Cys Leu Glu Asn Ser Ser Leu Glu His Thr Val His Arg Glu
65 70 75 80

Lys Thr Gly Lys Gly Leu Ser Ala Thr Arg Leu Ser Ala Ser Ser Glu
85 90 95

Asp Ile Ser Asp Arg Leu Ala Gly Val Ser Val Gly Leu Pro Ser Ser
100 105 110

Thr Thr Thr Glu Gln Pro Lys Pro Ala Val Gln Thr Lys Gly Arg Pro
115 120 125

His Ser Gln Cys Leu Asn Ser Ser Pro Leu Ser His Ala Gln Leu Met
130 135 140

Phe Pro Ala Pro Ser Ala Pro Cys Ser Ser Ala Pro Ser Val Pro Asp
145 150 155 160

Ile Ser Lys His Arg Pro Gln Ala Phe Val Pro Cys Lys Ile Pro Ser
165 170 175

Ala Ser Pro Gln Thr Gln Arg Lys Phe Ser Leu Gln Phe Gln Arg Asn
180 185 190

Cys Ser Glu His Arg Asp Ser Asp Gln Leu Ser Pro Val Phe Thr Gln
195 200 205

Ser Arg Pro Pro Pro Ser Ser Asn Ile His Arg Pro Lys Pro Ser Arg
210 215 220

Pro Val Pro Gly Ser Thr Ser Lys Leu Gly Asp Ala Thr Lys Ser Ser
225 230 235 240

Met Thr Leu Asp Leu Gly Ser Ala Ser Arg Cys Asp Asp Ser Phe Gly
245 250 255

Gly Gly Gly Asn Ser Gly Asn Ala Val Ile Pro Ser Asp Glu Thr Val
260 265 270

Phe Thr Pro Val Glu Asp Lys Cys Arg Leu Asp Val Asn Thr Glu Leu
275 280 285

Asn Ser Ser Ile Glu Asp Leu Leu Glu Ala Ser Met Pro Ser Ser Asp
290 295 300

Thr Thr Val Thr Phe Lys Ser Glu Val Ala Val Leu Ser Pro Glu Lys
305 310 315 320

Ala Glu Asn Asp Asp Thr Tyr Lys Asp Asp Val Asn His Asn Gln Lys
325 330 335

Cys Lys Glu Lys Met Glu Ala Glu Glu Glu Glu Ala Leu Ala Ile Ala

340 345 350

Met Ala Met Ser Ala Ser Gln Asp Ala Leu Pro Ile Val Pro Gln Leu
355 360 365

Gln Val Glu Asn Gly Glu Asp Ile Ile Ile Ile Gln Gln Asp Thr Pro
370 375 380

Glu Thr Leu Pro Gly His Thr Lys Ala Lys Gln Pro Tyr Arg Glu Asp
385 390 395 400

Ala Glu Trp Leu Lys Gly Gln Gln Ile Gly Leu Gly Ala Phe Ser Ser
405 410 415

Cys Tyr Gln Ala Gln Asp Val Gly Thr Gly Thr Leu Met Ala Val Lys
420 425 430

Gln Val Thr Tyr Val Arg Asn Thr Ser Ser Glu Gln Glu Glu Val Val
435 440 445

Glu Ala Leu Arg Glu Glu Ile Arg Met Met Gly His Leu Asn His Pro
450 455 460

Asn Ile Ile Arg Met Leu Gly Ala Thr Cys Glu Lys Ser Asn Tyr Asn
465 470 475 480

Leu Phe Ile Glu Trp Met Ala Gly Gly Ser Val Ala His Leu Leu Ser
485 490 495

Lys Tyr Gly Ala Phe Lys Glu Ser Val Val Ile Asn Tyr Thr Glu Gln
500 505 510

Leu Leu Arg Gly Leu Ser Tyr Leu His Glu Asn Gln Ile Ile His Arg
515 520 525

Asp Val Lys Gly Ala Asn Leu Leu Ile Asp Ser Thr Gly Gln Arg Leu
530 535 540

Arg Ile Ala Asp Phe Gly Ala Ala Ala Arg Leu Ala Ser Lys Gly Thr
545 550 555 560

Gly Ala Gly Glu Phe Gln Gly Gln Leu Leu Gly Thr Ile Ala Phe Met
565 570 575

Ala Pro Glu Val Leu Arg Gly Gln Gln Tyr Gly Arg Ser Cys Asp Val
580 585 590

Trp Ser Val Gly Cys Ala Ile Ile Glu Met Ala Cys Ala Lys Pro Pro
595 600 605

Trp Asn Ala Glu Lys His Ser Asn His Leu Ala Leu Ile Phe Lys Ile
610 615 620

Ala Ser Ala Thr Thr Ala Pro Ser Ile Pro Ser His Leu Ser Pro Gly
625 630 635 640

Leu Arg Asp Val Ala Val Arg Cys Leu Glu Leu Gln Pro Gln Asp Arg
645 650 655

Pro Pro Ser Arg Glu Leu Leu Lys His Pro Val Phe Arg Thr Thr Trp
660 665 670

<210> 3
<211> 5253
<212> DNA
<213> Murine

<220>
<221> CDS
<222> (15)..(4496)

<400> 3

```
gcccgcgaga gaaa atg gcg gcg gcg gcg ggc gat cgc gcc tcg tcg tcg   50
                Met Ala Ala Ala Ala Gly Asp Arg Ala Ser Ser Ser
                  1               5                  10

gga ttc ccg ggc gcc gcg gcg gcg agt ccc gag gcg ggc ggc ggc ggc   98
Gly Phe Pro Gly Ala Ala Ala Ala Ser Pro Glu Ala Gly Gly Gly Gly
        15                  20                  25

gga gga gga gga gct ctc cag gga agc ggc gcg ccc gca gcg ggc gcg   146
Gly Gly Gly Gly Ala Leu Gln Gly Ser Gly Ala Pro Ala Ala Gly Ala
     30                  35                  40

gcg ggg ctg ctg cgg gag cct ggc agc gcg ggc cgc gag cgc gcg gac   194
Ala Gly Leu Leu Arg Glu Pro Gly Ser Ala Gly Arg Glu Arg Ala Asp
 45                  50                  55                  60

tgg cgg cgg cgg cac gtg cgc aaa gtg cgg agt gtg gag ctg gac cag   242
Trp Arg Arg Arg His Val Arg Lys Val Arg Ser Val Glu Leu Asp Gln
                65                  70                  75

ctg ccg gag cag ccg ctc ttc ctc gcc gcc gcc tcg ccg ccc tgc cca   290
Leu Pro Glu Gln Pro Leu Phe Leu Ala Ala Ala Ser Pro Pro Cys Pro
            80                  85                  90

tct act tcc ccg tcg ccg gag ccc gcg gac gcg gct gca gga gcg agt   338
Ser Thr Ser Pro Ser Pro Glu Pro Ala Asp Ala Ala Ala Gly Ala Ser
        95                  100                 105

cgc ttc cag ccc gcg gcg gga ccg cca ccc ccg gga gcg gcg agt cgc   386
Arg Phe Gln Pro Ala Ala Gly Pro Pro Pro Pro Gly Ala Ala Ser Arg
    110                 115                 120

tgc ggc tcc cac tct gcc gag ctg gcg gcc gcg cgg gac agc ggc gcc   434
Cys Gly Ser His Ser Ala Glu Leu Ala Ala Ala Arg Asp Ser Gly Ala
125                 130                 135                 140

cgg agc ccc gcg ggg gcg gag ccg ccc tct gca gcg gcc ccc tcc ggt   482
Arg Ser Pro Ala Gly Ala Glu Pro Pro Ser Ala Ala Ala Pro Ser Gly
                145                 150                 155

cga gag atg gag aat aaa gaa acc ctc aaa gga ctg cac aag atg gag   530
Arg Glu Met Glu Asn Lys Glu Thr Leu Lys Gly Leu His Lys Met Glu
            160                 165                 170

gat cgc ccg gag gag aga atg atc cgg gag aag ctc aag gcg acc tgt   578
Asp Arg Pro Glu Glu Arg Met Ile Arg Glu Lys Leu Lys Ala Thr Cys
        175                 180                 185

atg ccg gcc tgg aag cac gag tgg ttg gag agg agg aac agg aga ggc   626
```

```
Met Pro Ala Trp Lys His Glu Trp Leu Glu Arg Arg Asn Arg Arg Gly
    190                 195                 200

cct gtg gtg gtg aag cca atc cct att aaa gga gat gga tct gaa gtg   674
Pro Val Val Val Lys Pro Ile Pro Ile Lys Gly Asp Gly Ser Glu Val
205                 210                 215                 220

aat aac ttg gca gct gag ccc cag gga gag ggc cag gca ggt tcc gct   722
Asn Asn Leu Ala Ala Glu Pro Gln Gly Glu Gly Gln Ala Gly Ser Ala
                225                 230                 235

gca cca gcc ccc aag ggc cga cga agc cca tct cct ggc agc tct ccg   770
Ala Pro Ala Pro Lys Gly Arg Arg Ser Pro Ser Pro Gly Ser Ser Pro
            240                 245                 250

tca ggg cgc tcg gtg aag ccg gaa tcc cca gga gta aga cgg aaa cga   818
Ser Gly Arg Ser Val Lys Pro Glu Ser Pro Gly Val Arg Arg Lys Arg
        255                 260                 265

gtg tcc ccg gtg cct ttc cag agt ggc aga atc aca cca ccc cga aga   866
Val Ser Pro Val Pro Phe Gln Ser Gly Arg Ile Thr Pro Pro Arg Arg
    270                 275                 280

gcc cca tca ccg gat ggc ttc tcc ccg tac agc cca gag gag acg agc   914
Ala Pro Ser Pro Asp Gly Phe Ser Pro Tyr Ser Pro Glu Glu Thr Ser
285                 290                 295                 300

cgc cgc gtg aac aaa gtg atg aga gcc agg ctg tac ctg ctg cag cag   962
Arg Arg Val Asn Lys Val Met Arg Ala Arg Leu Tyr Leu Leu Gln Gln
                305                 310                 315

ata gga ccc aac tct ttc ctg att gga gga gac agt cca gac aat aaa  1010
Ile Gly Pro Asn Ser Phe Leu Ile Gly Gly Asp Ser Pro Asp Asn Lys
            320                 325                 330

tac cgg gtg ttt att ggg cca cag aac tgc agc tgt ggg cgt gga gca  1058
Tyr Arg Val Phe Ile Gly Pro Gln Asn Cys Ser Cys Gly Arg Gly Ala
        335                 340                 345

ttc tgt att cac ctc ttg ttt gtc atg ctc cgg gtg ttt cag cta gaa  1106
Phe Cys Ile His Leu Leu Phe Val Met Leu Arg Val Phe Gln Leu Glu
    350                 355                 360

ccc tct gac ccc atg tta tgg aga aaa act tta aaa aat ttc gag gtt  1154
Pro Ser Asp Pro Met Leu Trp Arg Lys Thr Leu Lys Asn Phe Glu Val
365                 370                 375                 380

gag agt ttg ttc cag aaa tac cac agt agg cgt agc tcg aga atc aaa  1202
Glu Ser Leu Phe Gln Lys Tyr His Ser Arg Arg Ser Ser Arg Ile Lys
                385                 390                 395

gct cca tcc cgg aac acc atc cag aag ttt gtg tca cgc atg tca aat  1250
Ala Pro Ser Arg Asn Thr Ile Gln Lys Phe Val Ser Arg Met Ser Asn
            400                 405                 410

tct cac aca ctg tca tcg tct agc aca tcc aca tct agt tca gaa aac  1298
Ser His Thr Leu Ser Ser Ser Ser Thr Ser Thr Ser Ser Ser Glu Asn
        415                 420                 425

agc atc aag gat gaa gag gag cag atg tgt ccc atc tgc ttg ctg ggc  1346
Ser Ile Lys Asp Glu Glu Glu Gln Met Cys Pro Ile Cys Leu Leu Gly
```

```
    430                 435                 440

atg ctg gat gag gag agc ctg act gtg tgt gaa gat ggc tgc agg aac   1394
Met Leu Asp Glu Glu Ser Leu Thr Val Cys Glu Asp Gly Cys Arg Asn
445                 450                 455                 460

aag ctg cac cac cat tgc atg tcc atc tgg gcg gaa gag tgt aga aga   1442
Lys Leu His His His Cys Met Ser Ile Trp Ala Glu Glu Cys Arg Arg
                465                 470                 475

aat aga gag cct tta ata tgt ccc ctt tgt aga tct aag tgg aga tcc   1490
Asn Arg Glu Pro Leu Ile Cys Pro Leu Cys Arg Ser Lys Trp Arg Ser
            480                 485                 490

cat gac ttc tac agc cat gag tta tca agc ccc gtg gag tcc ccc gcc   1538
His Asp Phe Tyr Ser His Glu Leu Ser Ser Pro Val Glu Ser Pro Ala
        495                 500                 505

tcc ctg cga gct gtc cag cag cca tcc tcc ccg cag cag ccc gtg gcc   1586
Ser Leu Arg Ala Val Gln Gln Pro Ser Ser Pro Gln Gln Pro Val Ala
    510                 515                 520

gga tca cag cgg agg aat cag gag agc agt ttt aac ctt act cat ttt   1634
Gly Ser Gln Arg Arg Asn Gln Glu Ser Ser Phe Asn Leu Thr His Phe
525                 530                 535                 540

gga acc cag cag att cct tcc gct tac aaa gat ttg gcc gag cca tgg   1682
Gly Thr Gln Gln Ile Pro Ser Ala Tyr Lys Asp Leu Ala Glu Pro Trp
                545                 550                 555

att cag gtg ttt gga atg gaa ctc gtt ggc tgc tta ttc tct aga aac   1730
Ile Gln Val Phe Gly Met Glu Leu Val Gly Cys Leu Phe Ser Arg Asn
            560                 565                 570

tgg aac gta agg gaa atg gcc ctt agg cgt ctt tcc cac gac gtt agt   1778
Trp Asn Val Arg Glu Met Ala Leu Arg Arg Leu Ser His Asp Val Ser
        575                 580                 585

ggg gcc ctg ttg ttg gca aac ggg gag agc act gga aac tct gga ggc   1826
Gly Ala Leu Leu Leu Ala Asn Gly Glu Ser Thr Gly Asn Ser Gly Gly
    590                 595                 600

ggc agt ggg ggc agc tta agc gcg gga gcg gcc agc ggg tcc tcc cag   1874
Gly Ser Gly Gly Ser Leu Ser Ala Gly Ala Ala Ser Gly Ser Ser Gln
605                 610                 615                 620

ccc agc atc tca ggg gat gtg gtg gag gcg tgc tgc agt gtc ctg tct   1922
Pro Ser Ile Ser Gly Asp Val Val Glu Ala Cys Cys Ser Val Leu Ser
                625                 630                 635

ata gtc tgc gct gac cct gtc tac aaa gtg tac gtt gct gct tta aaa   1970
Ile Val Cys Ala Asp Pro Val Tyr Lys Val Tyr Val Ala Ala Leu Lys
            640                 645                 650

aca ttg aga gcc atg ctg gta tac act cct tgc cac agt ctg gca gaa   2018
Thr Leu Arg Ala Met Leu Val Tyr Thr Pro Cys His Ser Leu Ala Glu
        655                 660                 665

aga atc aaa ctt cag aga ctc ctc cgg cca gtt gta gac act atc ctt   2066
Arg Ile Lys Leu Gln Arg Leu Leu Arg Pro Val Val Asp Thr Ile Leu
    670                 675                 680
```

```
gtc aag tgt gca gat gcc aac agc cgc acg agt cag ctg tcc ata tct   2114
Val Lys Cys Ala Asp Ala Asn Ser Arg Thr Ser Gln Leu Ser Ile Ser
685                 690                 695                 700

aca gtg ctg gaa ctc tgc aag ggc caa gca gga gag ctg gcg gtt ggg   2162
Thr Val Leu Glu Leu Cys Lys Gly Gln Ala Gly Glu Leu Ala Val Gly
                705                 710                 715

aga gaa ata ctt aaa gct ggg tcc atc ggg gtt ggt ggt gtc gat tac   2210
Arg Glu Ile Leu Lys Ala Gly Ser Ile Gly Val Gly Gly Val Asp Tyr
            720                 725                 730

gtc tta agt tgt atc ctt gga aac caa gct gaa tca aac aac tgg caa   2258
Val Leu Ser Cys Ile Leu Gly Asn Gln Ala Glu Ser Asn Asn Trp Gln
        735                 740                 745

gaa ctg ctg ggt cgc ctc tgt ctt ata gac agg ttg ctg ttg gaa ttt   2306
Glu Leu Leu Gly Arg Leu Cys Leu Ile Asp Arg Leu Leu Leu Glu Phe
    750                 755                 760

cct gct gaa ttc tat cct cat att gtc agt act gat gtc tca caa gct   2354
Pro Ala Glu Phe Tyr Pro His Ile Val Ser Thr Asp Val Ser Gln Ala
765                 770                 775                 780

gag cct gtt gaa atc agg tac aag aag ctg ctc tcc ctc tta acc ttt   2402
Glu Pro Val Glu Ile Arg Tyr Lys Lys Leu Leu Ser Leu Leu Thr Phe
                785                 790                 795

gcc ttg caa tcc att gac aat tcc cac tcg atg gtt ggc aag ctc tct   2450
Ala Leu Gln Ser Ile Asp Asn Ser His Ser Met Val Gly Lys Leu Ser
            800                 805                 810

cgg agg ata tat ctg agc tct gcc agg atg gtg acc gca gtg ccc gct   2498
Arg Arg Ile Tyr Leu Ser Ser Ala Arg Met Val Thr Ala Val Pro Ala
        815                 820                 825

gtg ttt tcc aag ctg gta acc atg ctt aat gct tct ggc tcc acc cac   2546
Val Phe Ser Lys Leu Val Thr Met Leu Asn Ala Ser Gly Ser Thr His
    830                 835                 840

ttc acc agg atg cgc cgg cgt ctg atg gct atc gcg gat gag gta gaa   2594
Phe Thr Arg Met Arg Arg Arg Leu Met Ala Ile Ala Asp Glu Val Glu
845                 850                 855                 860

att gcc gag gtc atc cag ctg ggt gtg gag gac act gtg gat ggg cat   2642
Ile Ala Glu Val Ile Gln Leu Gly Val Glu Asp Thr Val Asp Gly His
                865                 870                 875

cag gac agc tta cag gcc gtg gcc ccc acc agc tgt cta gaa aac agc   2690
Gln Asp Ser Leu Gln Ala Val Ala Pro Thr Ser Cys Leu Glu Asn Ser
            880                 885                 890

tcc ctt gag cac aca gtc cat aga gag aaa act gga aaa gga cta agt   2738
Ser Leu Glu His Thr Val His Arg Glu Lys Thr Gly Lys Gly Leu Ser
        895                 900                 905

gct acg aga ctg agt gcc agc tcg gag gac att tct gac aga ctg gcc   2786
Ala Thr Arg Leu Ser Ala Ser Ser Glu Asp Ile Ser Asp Arg Leu Ala
    910                 915                 920
```

```
ggc gtc tct gta gga ctt ccc agc tca aca aca aca gaa caa cca aag   2834
Gly Val Ser Val Gly Leu Pro Ser Ser Thr Thr Thr Glu Gln Pro Lys
925                 930                 935                 940

cca gcg gtt caa aca aaa ggc aga ccc cac agt cag tgt ttg aac tcc   2882
Pro Ala Val Gln Thr Lys Gly Arg Pro His Ser Gln Cys Leu Asn Ser
                945                 950                 955

tcc cct ttg tct cat gct caa tta atg ttc cca gca cca tca gcc cct   2930
Ser Pro Leu Ser His Ala Gln Leu Met Phe Pro Ala Pro Ser Ala Pro
            960                 965                 970

tgt tcc tct gcc ccg tct gtc cca gat att tct aag cac aga ccc cag   2978
Cys Ser Ser Ala Pro Ser Val Pro Asp Ile Ser Lys His Arg Pro Gln
        975                 980                 985

gca ttt gtt ccc tgc aaa ata cct tcc gca tct cct cag aca cag cgc   3026
Ala Phe Val Pro Cys Lys Ile Pro Ser Ala Ser Pro Gln Thr Gln Arg
    990                 995                1000

aag ttc tct cta caa ttc cag agg aac tgc tct gaa cac cga gac tca   3074
Lys Phe Ser Leu Gln Phe Gln Arg Asn Cys Ser Glu His Arg Asp Ser
1005                1010                1015                1020

gac cag ctc tcc cca gtc ttc act cag tca aga ccc cca ccc tcc agt   3122
Asp Gln Leu Ser Pro Val Phe Thr Gln Ser Arg Pro Pro Pro Ser Ser
                1025                1030                1035

aac ata cac agg cca aag cca tcc cga ccc gtt ccg ggc agt aca agc   3170
Asn Ile His Arg Pro Lys Pro Ser Arg Pro Val Pro Gly Ser Thr Ser
            1040                1045                1050

aaa cta ggg gac gcc aca aaa agt agc atg aca ctt gat ctg ggc agt   3218
Lys Leu Gly Asp Ala Thr Lys Ser Ser Met Thr Leu Asp Leu Gly Ser
        1055                1060                1065

gct tcc agg tgt gac gac agc ttt ggc ggc ggc ggc aac agt ggc aac   3266
Ala Ser Arg Cys Asp Asp Ser Phe Gly Gly Gly Gly Asn Ser Gly Asn
    1070                1075                1080

gcc gtc ata ccc agc gac gag aca gtg ttc acg ccg gtg gag gac aag   3314
Ala Val Ile Pro Ser Asp Glu Thr Val Phe Thr Pro Val Glu Asp Lys
1085                1090                1095                1100

tgc agg tta gat gtg aac acc gag ctc aac tcc agc atc gag gac ctt   3362
Cys Arg Leu Asp Val Asn Thr Glu Leu Asn Ser Ser Ile Glu Asp Leu
                1105                1110                1115

ctt gaa gca tcc atg cct tca agt gac acg aca gtc act ttc aag tcc   3410
Leu Glu Ala Ser Met Pro Ser Ser Asp Thr Thr Val Thr Phe Lys Ser
            1120                1125                1130

gaa gtc gcc gtc ctc tct ccg gaa aag gcc gaa aat gac gac acc tac   3458
Glu Val Ala Val Leu Ser Pro Glu Lys Ala Glu Asn Asp Asp Thr Tyr
        1135                1140                1145

aaa gac gac gtc aat cat aat caa aag tgc aaa gaa aag atg gaa gct   3506
Lys Asp Asp Val Asn His Asn Gln Lys Cys Lys Glu Lys Met Glu Ala
    1150                1155                1160

gaa gag gag gag gct tta gcg atc gcc atg gcg atg tca gcg tct cag   3554
```

```
Glu Glu Glu Glu Ala Leu Ala Ile Ala Met Ala Met Ser Ala Ser Gln
1165                1170                1175                1180

gat gcc ctc ccc atc gtc cct cag ctg cag gtg gaa aat gga gaa gat   3602
Asp Ala Leu Pro Ile Val Pro Gln Leu Gln Val Glu Asn Gly Glu Asp
                1185                1190                1195

att atc atc att cag cag gac aca cca gaa act ctt cca gga cat acc   3650
Ile Ile Ile Ile Gln Gln Asp Thr Pro Glu Thr Leu Pro Gly His Thr
            1200                1205                1210

aaa gcg aaa cag cct tac aga gaa gac gct gag tgg ctg aaa ggc cag   3698
Lys Ala Lys Gln Pro Tyr Arg Glu Asp Ala Glu Trp Leu Lys Gly Gln
        1215                1220                1225

cag ata ggc ctc gga gca ttt tct tcc tgt tac caa gca cag gat gtg   3746
Gln Ile Gly Leu Gly Ala Phe Ser Ser Cys Tyr Gln Ala Gln Asp Val
    1230                1235                1240

ggg act ggg act tta atg gct gtg aaa cag gtg acg tac gtc aga aac   3794
Gly Thr Gly Thr Leu Met Ala Val Lys Gln Val Thr Tyr Val Arg Asn
1245                1250                1255                1260

aca tcc tcc gag cag gag gag gtg gtg gaa gcg ttg agg gaa gag atc   3842
Thr Ser Ser Glu Gln Glu Glu Val Val Glu Ala Leu Arg Glu Glu Ile
                1265                1270                1275

cgg atg atg ggt cac ctc aac cat cca aac atc atc cgg atg ctg ggg   3890
Arg Met Met Gly His Leu Asn His Pro Asn Ile Ile Arg Met Leu Gly
            1280                1285                1290

gcc acg tgc gag aag agc aac tac aac ctc ttc att gag tgg atg gcg   3938
Ala Thr Cys Glu Lys Ser Asn Tyr Asn Leu Phe Ile Glu Trp Met Ala
        1295                1300                1305

gga gga tct gtg gct cac ctc ttg agt aaa tac gga gct ttc aag gag   3986
Gly Gly Ser Val Ala His Leu Leu Ser Lys Tyr Gly Ala Phe Lys Glu
    1310                1315                1320

tca gtc gtc att aac tac act gag cag tta ctg cgt ggc ctt tcc tat   4034
Ser Val Val Ile Asn Tyr Thr Glu Gln Leu Leu Arg Gly Leu Ser Tyr
1325                1330                1335                1340

ctc cac gag aac cag atc att cac aga gac gtc aaa ggt gcc aac ctg   4082
Leu His Glu Asn Gln Ile Ile His Arg Asp Val Lys Gly Ala Asn Leu
                1345                1350                1355

ctc att gac agc acc ggt cag agg ctg aga att gca gac ttt gga gct   4130
Leu Ile Asp Ser Thr Gly Gln Arg Leu Arg Ile Ala Asp Phe Gly Ala
            1360                1365                1370

gct gcc agg ttg gca tca aaa gga acc ggt gca gga gag ttc cag gga   4178
Ala Ala Arg Leu Ala Ser Lys Gly Thr Gly Ala Gly Glu Phe Gln Gly
        1375                1380                1385

cag tta ctg ggg aca att gca ttc atg gcg cct gag gtc cta aga ggt   4226
Gln Leu Leu Gly Thr Ile Ala Phe Met Ala Pro Glu Val Leu Arg Gly
    1390                1395                1400

cag cag tat ggt agg agc tgt gat gta tgg agt gtt ggc tgc gcc att   4274
Gln Gln Tyr Gly Arg Ser Cys Asp Val Trp Ser Val Gly Cys Ala Ile
```

1405 1410 1415 1420

ata gaa atg gct tgt gca aaa cca cct tgg aat gca gaa aaa cac tcc 4322
Ile Glu Met Ala Cys Ala Lys Pro Pro Trp Asn Ala Glu Lys His Ser
1425 1430 1435

aat cat ctc gcc ttg ata ttt aag att gct agc gca act act gca ccg 4370
Asn His Leu Ala Leu Ile Phe Lys Ile Ala Ser Ala Thr Thr Ala Pro
1440 1445 1450

tcc atc ccg tca cac ctg tcc ccg ggt ctg cgc gac gtg gcc gtg cgc 4418
Ser Ile Pro Ser His Leu Ser Pro Gly Leu Arg Asp Val Ala Val Arg
1455 1460 1465

tgc tta gaa ctt cag cct cag gac cgg cct ccg tcc aga gag ctg ctg 4466
Cys Leu Glu Leu Gln Pro Gln Asp Arg Pro Pro Ser Arg Glu Leu Leu
1470 1475 1480

aaa cat ccg gtc ttc cgt acc acg tgg tag ttaattgttc agatcagctc 4516
Lys His Pro Val Phe Arg Thr Thr Trp
1485 1490

taatggagac aggatatgca accgggagag agaaaagaga acttgtgggc gaccatgccg 4576

ctaaccgcag ccctcacgcc actgaacagc cagaaacggg gccagcgggg aaccgtacct 4636

aagcatgtga ttgacaaatc atgacctgta cctaagctcg atatgcagac atctacagct 4696

cgtgcaggaa ctgcacaccg tgcctttcac aggactggct ctgggggacc aggaaggcga 4756

tggagtttgc atgactaaag aacagaagca taaatttatt tttggagcac tttttcagct 4816

aatcagtatt accatgtaca tcaacatgcc cgccacattt caaactcaga ctgtcccaga 4876

tgtcaagatc cactgtgttt gagtttgttt gcagttccct cagcttgctg gtaattgtgg 4936

tgttttgttt tcgatgcaaa tgtgatgtaa tattcttatt ttctttggat caaagctgga 4996

ctgaaaattg tactgtgtaa ttatttttgt gtttttaatg ttatttggta ctcgaattgt 5056

aaataacgtc tactgctgtt tattccagtt tctactacct caggtgtcct atagattttt 5116

cttctaccaa agttcactct cagaatgaaa ttctacgtgc tgtgtgacta tgactcctaa 5176

gacttccagg gcttaagggc taactcctat tagcacctta ctatgtaagc aaatgctaca 5236

aaaaaaaaaa aaaaaaa 5253

<210> 4
<211> 1493
<212> PRT
<213> Murine

<400> 4
Met Ala Ala Ala Ala Gly Asp Arg Ala Ser Ser Ser Gly Phe Pro Gly
1 5 10 15

Ala Ala Ala Ala Ser Pro Glu Ala Gly Gly Gly Gly Gly Gly Gly Gly
20 25 30

Ala Leu Gln Gly Ser Gly Ala Pro Ala Ala Gly Ala Ala Gly Leu Leu
35 40 45

Arg Glu Pro Gly Ser Ala Gly Arg Glu Arg Ala Asp Trp Arg Arg Arg
50 55 60

His Val Arg Lys Val Arg Ser Val Glu Leu Asp Gln Leu Pro Glu Gln
65 70 75 80

Pro Leu Phe Leu Ala Ala Ala Ser Pro Pro Cys Pro Ser Thr Ser Pro
85 90 95

Ser Pro Glu Pro Ala Asp Ala Ala Ala Gly Ala Ser Arg Phe Gln Pro
100 105 110

Ala Ala Gly Pro Pro Pro Pro Gly Ala Ala Ser Arg Cys Gly Ser His
115 120 125

Ser Ala Glu Leu Ala Ala Ala Arg Asp Ser Gly Ala Arg Ser Pro Ala
130 135 140

Gly Ala Glu Pro Pro Ser Ala Ala Ala Pro Ser Gly Arg Glu Met Glu
145 150 155 160

Asn Lys Glu Thr Leu Lys Gly Leu His Lys Met Glu Asp Arg Pro Glu
165 170 175

Glu Arg Met Ile Arg Glu Lys Leu Lys Ala Thr Cys Met Pro Ala Trp
180 185 190

Lys His Glu Trp Leu Glu Arg Arg Asn Arg Arg Gly Pro Val Val Val
195 200 205

Lys Pro Ile Pro Ile Lys Gly Asp Gly Ser Glu Val Asn Asn Leu Ala
210 215 220

Ala Glu Pro Gln Gly Glu Gly Gln Ala Gly Ser Ala Ala Pro Ala Pro
225 230 235 240

Lys Gly Arg Arg Ser Pro Ser Pro Gly Ser Ser Pro Ser Gly Arg Ser
245 250 255

Val Lys Pro Glu Ser Pro Gly Val Arg Arg Lys Arg Val Ser Pro Val
260 265 270

Pro Phe Gln Ser Gly Arg Ile Thr Pro Pro Arg Arg Ala Pro Ser Pro
275 280 285

Asp Gly Phe Ser Pro Tyr Ser Pro Glu Glu Thr Ser Arg Arg Val Asn
290 295 300

Lys Val Met Arg Ala Arg Leu Tyr Leu Leu Gln Gln Ile Gly Pro Asn
305 310 315 320

Ser Phe Leu Ile Gly Gly Asp Ser Pro Asp Asn Lys Tyr Arg Val Phe
325 330 335

Ile Gly Pro Gln Asn Cys Ser Cys Gly Arg Gly Ala Phe Cys Ile His
340 345 350

Leu Leu Phe Val Met Leu Arg Val Phe Gln Leu Glu Pro Ser Asp Pro

355 360 365

Met Leu Trp Arg Lys Thr Leu Lys Asn Phe Glu Val Glu Ser Leu Phe
370 375 380

Gln Lys Tyr His Ser Arg Arg Ser Ser Arg Ile Lys Ala Pro Ser Arg
385 390 395 400

Asn Thr Ile Gln Lys Phe Val Ser Arg Met Ser Asn Ser His Thr Leu
405 410 415

Ser Ser Ser Ser Thr Ser Thr Ser Ser Ser Glu Asn Ser Ile Lys Asp
420 425 430

Glu Glu Glu Gln Met Cys Pro Ile Cys Leu Leu Gly Met Leu Asp Glu
435 440 445

Glu Ser Leu Thr Val Cys Glu Asp Gly Cys Arg Asn Lys Leu His His
450 455 460

His Cys Met Ser Ile Trp Ala Glu Glu Cys Arg Arg Asn Arg Glu Pro
465 470 475 480

Leu Ile Cys Pro Leu Cys Arg Ser Lys Trp Arg Ser His Asp Phe Tyr
485 490 495

Ser His Glu Leu Ser Ser Pro Val Glu Ser Pro Ala Ser Leu Arg Ala
500 505 510

Val Gln Gln Pro Ser Ser Pro Gln Gln Pro Val Ala Gly Ser Gln Arg
515 520 525

Arg Asn Gln Glu Ser Ser Phe Asn Leu Thr His Phe Gly Thr Gln Gln
530 535 540

Ile Pro Ser Ala Tyr Lys Asp Leu Ala Glu Pro Trp Ile Gln Val Phe
545 550 555 560

Gly Met Glu Leu Val Gly Cys Leu Phe Ser Arg Asn Trp Asn Val Arg
565 570 575

Glu Met Ala Leu Arg Arg Leu Ser His Asp Val Ser Gly Ala Leu Leu
580 585 590

Leu Ala Asn Gly Glu Ser Thr Gly Asn Ser Gly Gly Gly Ser Gly Gly
595 600 605

Ser Leu Ser Ala Gly Ala Ala Ser Gly Ser Ser Gln Pro Ser Ile Ser
610 615 620

Gly Asp Val Val Glu Ala Cys Cys Ser Val Leu Ser Ile Val Cys Ala
625 630 635 640

Asp Pro Val Tyr Lys Val Tyr Val Ala Ala Leu Lys Thr Leu Arg Ala
645 650 655

Met Leu Val Tyr Thr Pro Cys His Ser Leu Ala Glu Arg Ile Lys Leu
660 665 670

Gln Arg Leu Leu Arg Pro Val Val Asp Thr Ile Leu Val Lys Cys Ala
675 680 685

Asp Ala Asn Ser Arg Thr Ser Gln Leu Ser Ile Ser Thr Val Leu Glu
690 695 700

Leu Cys Lys Gly Gln Ala Gly Glu Leu Ala Val Gly Arg Glu Ile Leu
705 710 715 720

Lys Ala Gly Ser Ile Gly Val Gly Gly Val Asp Tyr Val Leu Ser Cys
725 730 735

Ile Leu Gly Asn Gln Ala Glu Ser Asn Asn Trp Gln Glu Leu Leu Gly
740 745 750

Arg Leu Cys Leu Ile Asp Arg Leu Leu Leu Glu Phe Pro Ala Glu Phe
755 760 765

Tyr Pro His Ile Val Ser Thr Asp Val Ser Gln Ala Glu Pro Val Glu
770 775 780

Ile Arg Tyr Lys Lys Leu Leu Ser Leu Leu Thr Phe Ala Leu Gln Ser
785 790 795 800

Ile Asp Asn Ser His Ser Met Val Gly Lys Leu Ser Arg Arg Ile Tyr
805 810 815

Leu Ser Ser Ala Arg Met Val Thr Ala Val Pro Ala Val Phe Ser Lys
820 825 830

Leu Val Thr Met Leu Asn Ala Ser Gly Ser Thr His Phe Thr Arg Met
835 840 845

Arg Arg Arg Leu Met Ala Ile Ala Asp Glu Val Glu Ile Ala Glu Val
850 855 860

Ile Gln Leu Gly Val Glu Asp Thr Val Asp Gly His Gln Asp Ser Leu
865 870 875 880

Gln Ala Val Ala Pro Thr Ser Cys Leu Glu Asn Ser Ser Leu Glu His
885 890 895

Thr Val His Arg Glu Lys Thr Gly Lys Gly Leu Ser Ala Thr Arg Leu
900 905 910

Ser Ala Ser Ser Glu Asp Ile Ser Asp Arg Leu Ala Gly Val Ser Val
915 920 925

Gly Leu Pro Ser Ser Thr Thr Thr Glu Gln Pro Lys Pro Ala Val Gln
930 935 940

Thr Lys Gly Arg Pro His Ser Gln Cys Leu Asn Ser Ser Pro Leu Ser
945 950 955 960

His Ala Gln Leu Met Phe Pro Ala Pro Ser Ala Pro Cys Ser Ser Ala
965 970 975

Pro Ser Val Pro Asp Ile Ser Lys His Arg Pro Gln Ala Phe Val Pro
980 985 990

Cys Lys Ile Pro Ser Ala Ser Pro Gln Thr Gln Arg Lys Phe Ser Leu
995 1000 1005

Gln Phe Gln Arg Asn Cys Ser Glu His Arg Asp Ser Asp Gln Leu Ser
1010 1015 1020

Pro Val Phe Thr Gln Ser Arg Pro Pro Pro Ser Ser Asn Ile His Arg
025 1030 1035 1040

Pro Lys Pro Ser Arg Pro Val Pro Gly Ser Thr Ser Lys Leu Gly Asp
1045 1050 1055

Ala Thr Lys Ser Ser Met Thr Leu Asp Leu Gly Ser Ala Ser Arg Cys
1060 1065 1070

Asp Asp Ser Phe Gly Gly Gly Gly Asn Ser Gly Asn Ala Val Ile Pro
1075 1080 1085

Ser Asp Glu Thr Val Phe Thr Pro Val Glu Asp Lys Cys Arg Leu Asp
1090 1095 1100

Val Asn Thr Glu Leu Asn Ser Ser Ile Glu Asp Leu Leu Glu Ala Ser
105 1110 1115 1120

Met Pro Ser Ser Asp Thr Thr Val Thr Phe Lys Ser Glu Val Ala Val
1125 1130 1135

Leu Ser Pro Glu Lys Ala Glu Asn Asp Asp Thr Tyr Lys Asp Asp Val
1140 1145 1150

Asn His Asn Gln Lys Cys Lys Glu Lys Met Glu Ala Glu Glu Glu Glu
1155 1160 1165

Ala Leu Ala Ile Ala Met Ala Met Ser Ala Ser Gln Asp Ala Leu Pro
1170 1175 1180

Ile Val Pro Gln Leu Gln Val Glu Asn Gly Glu Asp Ile Ile Ile Ile
185 1190 1195 1200

Gln Gln Asp Thr Pro Glu Thr Leu Pro Gly His Thr Lys Ala Lys Gln
1205 1210 1215

Pro Tyr Arg Glu Asp Ala Glu Trp Leu Lys Gly Gln Gln Ile Gly Leu
1220 1225 1230

Gly Ala Phe Ser Ser Cys Tyr Gln Ala Gln Asp Val Gly Thr Gly Thr
1235 1240 1245

Leu Met Ala Val Lys Gln Val Thr Tyr Val Arg Asn Thr Ser Ser Glu
1250 1255 1260

Gln Glu Glu Val Val Glu Ala Leu Arg Glu Glu Ile Arg Met Met Gly
265 1270 1275 1280

His Leu Asn His Pro Asn Ile Ile Arg Met Leu Gly Ala Thr Cys Glu
1285 1290 1295

Lys Ser Asn Tyr Asn Leu Phe Ile Glu Trp Met Ala Gly Gly Ser Val
1300 1305 1310

Ala His Leu Leu Ser Lys Tyr Gly Ala Phe Lys Glu Ser Val Val Ile
1315 1320 1325

Asn Tyr Thr Glu Gln Leu Leu Arg Gly Leu Ser Tyr Leu His Glu Asn

1330 1335 1340

Gln Ile Ile His Arg Asp Val Lys Gly Ala Asn Leu Leu Ile Asp Ser
345 1350 1355 1360

Thr Gly Gln Arg Leu Arg Ile Ala Asp Phe Gly Ala Ala Ala Arg Leu
1365 1370 1375

Ala Ser Lys Gly Thr Gly Ala Gly Glu Phe Gln Gly Gln Leu Leu Gly
1380 1385 1390

Thr Ile Ala Phe Met Ala Pro Glu Val Leu Arg Gly Gln Gln Tyr Gly
1395 1400 1405

Arg Ser Cys Asp Val Trp Ser Val Gly Cys Ala Ile Ile Glu Met Ala
1410 1415 1420

Cys Ala Lys Pro Pro Trp Asn Ala Glu Lys His Ser Asn His Leu Ala
425 1430 1435 1440

Leu Ile Phe Lys Ile Ala Ser Ala Thr Thr Ala Pro Ser Ile Pro Ser
1445 1450 1455

His Leu Ser Pro Gly Leu Arg Asp Val Ala Val Arg Cys Leu Glu Leu
1460 1465 1470

Gln Pro Gln Asp Arg Pro Pro Ser Arg Glu Leu Leu Lys His Pro Val
1475 1480 1485

Phe Arg Thr Thr Trp
1490

<210> 5
<211> 3911
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (3)..(3911)

<400> 5
cg gcc tgg aag cac gag tgg ttg gaa agg aga aat agg cga ggg cct 47
Ala Trp Lys His Glu Trp Leu Glu Arg Arg Asn Arg Arg Gly Pro
1 5 10 15

gtg gtg gta aaa cca atc cca gtt aaa gga gat gga tct gaa atg aat 95
Val Val Val Lys Pro Ile Pro Val Lys Gly Asp Gly Ser Glu Met Asn
20 25 30

cac tta gca gct gag tct cca gga gag gtc cag gca agt gcg gct tca 143
His Leu Ala Ala Glu Ser Pro Gly Glu Val Gln Ala Ser Ala Ala Ser
35 40 45

cca gct tcc aaa ggc cga cgc agt cct tct cct ggc aac tcc cca tca 191
Pro Ala Ser Lys Gly Arg Arg Ser Pro Ser Pro Gly Asn Ser Pro Ser
50 55 60

ggt cgc aca gtg aaa tca gaa tct cca gga gta agg aga aaa aga gtt 239
Gly Arg Thr Val Lys Ser Glu Ser Pro Gly Val Arg Arg Lys Arg Val

```
     65                  70                  75

tcc cca gtg cct ttt cag agt ggc aga atc aca cca ccc cga aga gcc   287
Ser Pro Val Pro Phe Gln Ser Gly Arg Ile Thr Pro Pro Arg Arg Ala
 80                  85                  90                  95

cct tca cca gat ggc ttc tca cca tat agc cct gag gaa aca aac cgc   335
Pro Ser Pro Asp Gly Phe Ser Pro Tyr Ser Pro Glu Glu Thr Asn Arg
                100                 105                 110

cgt gtt aac aaa gtg atg cgg gcc aga ctg tac tta ctg cag cag ata   383
Arg Val Asn Lys Val Met Arg Ala Arg Leu Tyr Leu Leu Gln Gln Ile
            115                 120                 125

ggg cct aac tct ttc ctg att gga gga gac agc cca gac aat aaa tac   431
Gly Pro Asn Ser Phe Leu Ile Gly Gly Asp Ser Pro Asp Asn Lys Tyr
        130                 135                 140

cgg gtg ttt att ggg cct cag aac tgc agc tgt gca cgt gga aca ttc   479
Arg Val Phe Ile Gly Pro Gln Asn Cys Ser Cys Ala Arg Gly Thr Phe
    145                 150                 155

tgt att cat ctg cta ttt gtg atg ctc cgg gtg ttt caa cta gaa cct   527
Cys Ile His Leu Leu Phe Val Met Leu Arg Val Phe Gln Leu Glu Pro
160                 165                 170                 175

tca gac cca atg tta tgg aga aaa act tta aag aat ttt gag gtt gag   575
Ser Asp Pro Met Leu Trp Arg Lys Thr Leu Lys Asn Phe Glu Val Glu
                180                 185                 190

agt ttg ttc cag aaa tat cac agt agg cgt agc tca agg atc aaa gct   623
Ser Leu Phe Gln Lys Tyr His Ser Arg Arg Ser Ser Arg Ile Lys Ala
            195                 200                 205

cca tct cgt aac acc atc cag aag ttt gtt tca cgc atg tca aat tct   671
Pro Ser Arg Asn Thr Ile Gln Lys Phe Val Ser Arg Met Ser Asn Ser
        210                 215                 220

cat aca ttg tca tca tct agt act tct aca tct agt tca gta aac agc   719
His Thr Leu Ser Ser Ser Ser Thr Ser Thr Ser Ser Ser Val Asn Ser
    225                 230                 235

ata aag gat gaa gag gaa cag atg tgt cct att tgc ttg ttg ggc atg   767
Ile Lys Asp Glu Glu Glu Gln Met Cys Pro Ile Cys Leu Leu Gly Met
240                 245                 250                 255

ctt gat gaa gaa agt ctt aca gtg tgt gaa gac ggc tgc agg aac aag   815
Leu Asp Glu Glu Ser Leu Thr Val Cys Glu Asp Gly Cys Arg Asn Lys
                260                 265                 270

ctg cac cac cac tgc atg tca att tgg gca gaa gag tgt aga aga aat   863
Leu His His His Cys Met Ser Ile Trp Ala Glu Glu Cys Arg Arg Asn
            275                 280                 285

aga gaa cct tta ata tgt ccc ctt tgt aga tct aag tgg aga tct cat   911
Arg Glu Pro Leu Ile Cys Pro Leu Cys Arg Ser Lys Trp Arg Ser His
        290                 295                 300

gat ttc tac agc cac gag ttg tca agt cct gtg gat tcc cct tct tcc   959
Asp Phe Tyr Ser His Glu Leu Ser Ser Pro Val Asp Ser Pro Ser Ser
    305                 310                 315
```

```
ctc aga gct gca cag cag caa acc gta cag cag cag cct ttg gct gga   1007
Leu Arg Ala Ala Gln Gln Gln Thr Val Gln Gln Gln Pro Leu Ala Gly
320                 325                 330                 335

tca cga agg aat caa gag agc aat ttt aac ctt act cat tat gga act   1055
Ser Arg Arg Asn Gln Glu Ser Asn Phe Asn Leu Thr His Tyr Gly Thr
                340                 345                 350

cag caa atc cct cct gct tac aaa gat tta gct gag cca tgg att cag   1103
Gln Gln Ile Pro Pro Ala Tyr Lys Asp Leu Ala Glu Pro Trp Ile Gln
            355                 360                 365

gtg ttt gga atg gaa ctc gtt ggc tgc tta ttc tct aga aac tgg aac   1151
Val Phe Gly Met Glu Leu Val Gly Cys Leu Phe Ser Arg Asn Trp Asn
        370                 375                 380

gta agg gaa atg gcc ctt agg cgt ctt tcc cac gac gtt agt ggg gcc   1199
Val Arg Glu Met Ala Leu Arg Arg Leu Ser His Asp Val Ser Gly Ala
    385                 390                 395

ctg ttg ttg gca aac ggg gag agc act gga aac tct gga ggc ggc agt   1247
Leu Leu Leu Ala Asn Gly Glu Ser Thr Gly Asn Ser Gly Gly Gly Ser
400                 405                 410                 415

ggg ggc agc tta agc gcg gga gcg gcc agc ggg tcc tcc cag ccc agc   1295
Gly Gly Ser Leu Ser Ala Gly Ala Ala Ser Gly Ser Ser Gln Pro Ser
                420                 425                 430

atc tca ggg gat gtg gtg gag gcg tgc tgc agt gtc ctg tct ata gtc   1343
Ile Ser Gly Asp Val Val Glu Ala Cys Cys Ser Val Leu Ser Ile Val
            435                 440                 445

tgc gct gac cct gtc tac aaa gtg tac gtt gct gct tta aaa aca ttg   1391
Cys Ala Asp Pro Val Tyr Lys Val Tyr Val Ala Ala Leu Lys Thr Leu
        450                 455                 460

aga gcc atg ctg gta tac act cct tgc cac agt ctg gca gaa aga atc   1439
Arg Ala Met Leu Val Tyr Thr Pro Cys His Ser Leu Ala Glu Arg Ile
    465                 470                 475

aaa ctt cag aga ctc ctc cgg cca gtt gta gac act atc ctt gtc aag   1487
Lys Leu Gln Arg Leu Leu Arg Pro Val Val Asp Thr Ile Leu Val Lys
480                 485                 490                 495

tgt gca gat gcc aac agc cgc acg agt cag ctg tcc ata tct aca gtg   1535
Cys Ala Asp Ala Asn Ser Arg Thr Ser Gln Leu Ser Ile Ser Thr Val
                500                 505                 510

ctg gaa ctc tgc aat ggc caa gca gga aag ctg gcg gtt ggg aga gaa   1583
Leu Glu Leu Cys Asn Gly Gln Ala Gly Lys Leu Ala Val Gly Arg Glu
            515                 520                 525

ata ctt aaa gct ggg tcc atc ggg gtt ggt ggt gtc gat tac gtc tta   1631
Ile Leu Lys Ala Gly Ser Ile Gly Val Gly Gly Val Asp Tyr Val Leu
        530                 535                 540

agt tgt atc ctt gga aac caa gct gaa tca aac aac tgg caa gaa ctg   1679
Ser Cys Ile Leu Gly Asn Gln Ala Glu Ser Asn Asn Trp Gln Glu Leu
    545                 550                 555
```

```
ctg ggt cgc ctc tgt ctt ata gac agg ttg ctg ttg gaa ttt cct gct   1727
Leu Gly Arg Leu Cys Leu Ile Asp Arg Leu Leu Leu Glu Phe Pro Ala
560                 565                 570                 575

gaa ttc tat cct cat att gtc agt act gat gtc tca caa gct gag cct   1775
Glu Phe Tyr Pro His Ile Val Ser Thr Asp Val Ser Gln Ala Glu Pro
                580                 585                 590

gtt gaa atc agg tac aag aag ctg ctc tcc ctc tta acc ttt gcc ttg   1823
Val Glu Ile Arg Tyr Lys Lys Leu Leu Ser Leu Leu Thr Phe Ala Leu
            595                 600                 605

caa tcc att gac aat tcc cac tcg atg gtt ggc aag ctc tct cgg agg   1871
Gln Ser Ile Asp Asn Ser His Ser Met Val Gly Lys Leu Ser Arg Arg
        610                 615                 620

ata tat ctg agc tct gcc agg atg gtg acc gca gtg ccc gct gtg ttt   1919
Ile Tyr Leu Ser Ser Ala Arg Met Val Thr Ala Val Pro Ala Val Phe
    625                 630                 635

tcc aag ctg gta acc atg ctt aat gct tct ggc tcc acc cac ttc acc   1967
Ser Lys Leu Val Thr Met Leu Asn Ala Ser Gly Ser Thr His Phe Thr
640                 645                 650                 655

agg atg cgc cgg cgt ctg atg gct atc gcg gat gag gta gaa att gcc   2015
Arg Met Arg Arg Arg Leu Met Ala Ile Ala Asp Glu Val Glu Ile Ala
                660                 665                 670

gag gtc atc cag ctg ggt gtg gag gac act gtg gat ggg cat cag gac   2063
Glu Val Ile Gln Leu Gly Val Glu Asp Thr Val Asp Gly His Gln Asp
            675                 680                 685

agc tta cag gcg ctg gcc ccc gcc agc tgt cta gaa aac agc tcc ctt   2111
Ser Leu Gln Ala Leu Ala Pro Ala Ser Cys Leu Glu Asn Ser Ser Leu
        690                 695                 700

gag cac aca gtc cat aga gag aaa act gga aaa gga cta agt gct acg   2159
Glu His Thr Val His Arg Glu Lys Thr Gly Lys Gly Leu Ser Ala Thr
    705                 710                 715

aga ctg agt gcc agc tcg gag gac att tct gac aga ctg gcc ggc gtc   2207
Arg Leu Ser Ala Ser Ser Glu Asp Ile Ser Asp Arg Leu Ala Gly Val
720                 725                 730                 735

tct gta gga ctt ccc agc tca aca aca aca gaa caa cca aag cca gcg   2255
Ser Val Gly Leu Pro Ser Ser Thr Thr Thr Glu Gln Pro Lys Pro Ala
                740                 745                 750

gtt caa aca aaa ggc aga ccc cac agt cag tgt ttg aac tcc tcc cct   2303
Val Gln Thr Lys Gly Arg Pro His Ser Gln Cys Leu Asn Ser Ser Pro
            755                 760                 765

ttg tct cat gct caa tta atg ttc cca gca cca tca gcc cct tgt tcc   2351
Leu Ser His Ala Gln Leu Met Phe Pro Ala Pro Ser Ala Pro Cys Ser
        770                 775                 780

tct gcc ccg tct gtc cca gat att tct aag cac aga ccc cag gca ttt   2399
Ser Ala Pro Ser Val Pro Asp Ile Ser Lys His Arg Pro Gln Ala Phe
    785                 790                 795

gtt ccc tgc aaa ata cct tcc gca tct cct cag aca cag cgc aag ttc   2447
```

```
Val Pro Cys Lys Ile Pro Ser Ala Ser Pro Gln Thr Gln Arg Lys Phe
800                 805                 810                 815

tct cta caa ttc cag agg aac tgc tct gaa cac cga gac tca gac cag   2495
Ser Leu Gln Phe Gln Arg Asn Cys Ser Glu His Arg Asp Ser Asp Gln
                820                 825                 830

ctc tcc cca gtc ttc act cag tca aga ccc cca ccc tcc agt aac ata   2543
Leu Ser Pro Val Phe Thr Gln Ser Arg Pro Pro Pro Ser Ser Asn Ile
            835                 840                 845

cac agg cca aag cca tcc cga ccc gtt ccg ggc agt aca agc aaa cta   2591
His Arg Pro Lys Pro Ser Arg Pro Val Pro Gly Ser Thr Ser Lys Leu
        850                 855                 860

ggg gac gcc aca aaa agt agc atg aca ctt gat ctg ggc agt gct tcc   2639
Gly Asp Ala Thr Lys Ser Ser Met Thr Leu Asp Leu Gly Ser Ala Ser
    865                 870                 875

agg tgt gac gac agc ttt ggc ggc ggc ggc aac agt ggc aac gcc gtc   2687
Arg Cys Asp Asp Ser Phe Gly Gly Gly Gly Asn Ser Gly Asn Ala Val
880                 885                 890                 895

ata ccc agc gac gag aca gtg ttc acg ccg gtg gag gac aag tgc agg   2735
Ile Pro Ser Asp Glu Thr Val Phe Thr Pro Val Glu Asp Lys Cys Arg
                900                 905                 910

tta gat gtg aac acc gag ctc aac tcc agc atc gag gac ctt ctt gaa   2783
Leu Asp Val Asn Thr Glu Leu Asn Ser Ser Ile Glu Asp Leu Leu Glu
            915                 920                 925

gca tcc atg cct tca agt gac acg aca gtc act ttc aag tcc gaa gtc   2831
Ala Ser Met Pro Ser Ser Asp Thr Thr Val Thr Phe Lys Ser Glu Val
        930                 935                 940

gcc gtc ctc tct ccg gaa aag gcc gaa aat gac gac acc tac aaa gac   2879
Ala Val Leu Ser Pro Glu Lys Ala Glu Asn Asp Asp Thr Tyr Lys Asp
    945                 950                 955

gac gtc aat cat aat caa aag tgc aaa gaa aag atg gaa gct gaa gag   2927
Asp Val Asn His Asn Gln Lys Cys Lys Glu Lys Met Glu Ala Glu Glu
960                 965                 970                 975

gag gag gct tta gcg atc gcc atg gcg atg tca gcg tct cag gat gcc   2975
Glu Glu Ala Leu Ala Ile Ala Met Ala Met Ser Ala Ser Gln Asp Ala
                980                 985                 990

ctc ccc atc gtc cct cag ctg cag gtg gaa aat gga gaa gat att atc   3023
Leu Pro Ile Val Pro Gln Leu Gln Val Glu Asn Gly Glu Asp Ile Ile
            995                1000                1005

atc att cag cag gac aca cca gaa act ctt cca gga cat acc aaa gcg   3071
Ile Ile Gln Gln Asp Thr Pro Glu Thr Leu Pro Gly His Thr Lys Ala
       1010                1015                1020

aaa cag cct tac aga gaa gac gct gag tgg ctg aaa ggc cag cag ata   3119
Lys Gln Pro Tyr Arg Glu Asp Ala Glu Trp Leu Lys Gly Gln Gln Ile
   1025                1030                1035

ggc ctc gga gca ttt tct tct tgt tat cag gct caa gat gtg gga act   3167
Gly Leu Gly Ala Phe Ser Ser Cys Tyr Gln Ala Gln Asp Val Gly Thr
```

```
1040                1045                1050                1055

gga act tta atg gct gtt aaa cag gtg act tat gtc aga aac aca tct   3215
Gly Thr Leu Met Ala Val Lys Gln Val Thr Tyr Val Arg Asn Thr Ser
                1060                1065                1070

tct gag caa gaa gaa gta gta gaa gca cta aga gaa gag ata aga atg   3263
Ser Glu Gln Glu Glu Val Val Glu Ala Leu Arg Glu Glu Ile Arg Met
            1075                1080                1085

atg agc cat ctg aat cat cca aac atc att agg atg ttg gga gcc acg   3311
Met Ser His Leu Asn His Pro Asn Ile Ile Arg Met Leu Gly Ala Thr
        1090                1095                1100

tgt gag aag agc aat tac aat ctc ttc att gaa tgg atg gca ggg gga   3359
Cys Glu Lys Ser Asn Tyr Asn Leu Phe Ile Glu Trp Met Ala Gly Gly
    1105                1110                1115

tcg gtg gct cat ttg ctg agt aaa tat gga gcc ttc aaa gaa tca gta   3407
Ser Val Ala His Leu Leu Ser Lys Tyr Gly Ala Phe Lys Glu Ser Val
1120                1125                1130                1135

gtt att aac tac act gaa cag tta ctc cgt ggc ctt tcg tat ctc cat   3455
Val Ile Asn Tyr Thr Glu Gln Leu Leu Arg Gly Leu Ser Tyr Leu His
                1140                1145                1150

gag aac cag atc att cac aga gat gtc aaa ggt gcc aat ttg ctc att   3503
Glu Asn Gln Ile Ile His Arg Asp Val Lys Gly Ala Asn Leu Leu Ile
            1155                1160                1165

gac agc acc ggt cag agg ctg aga att gca gac ttt gga gct gca gcc   3551
Asp Ser Thr Gly Gln Arg Leu Arg Ile Ala Asp Phe Gly Ala Ala Ala
        1170                1175                1180

agg ttg gca tca aaa gga act ggt gca gga gag ttt cag gga caa tta   3599
Arg Leu Ala Ser Lys Gly Thr Gly Ala Gly Glu Phe Gln Gly Gln Leu
    1185                1190                1195

ctg ggg aca att gca ttc atg gcg cct gag gtc cta aga ggt cag cag   3647
Leu Gly Thr Ile Ala Phe Met Ala Pro Glu Val Leu Arg Gly Gln Gln
1200                1205                1210                1215

tat ggt agg agc tgt gat gta tgg agt gtt ggc tgc gcc att ata gaa   3695
Tyr Gly Arg Ser Cys Asp Val Trp Ser Val Gly Cys Ala Ile Ile Glu
                1220                1225                1230

atg gct tgt gca aaa cca cct tgg aat gca gaa aaa cac tcc aat cat   3743
Met Ala Cys Ala Lys Pro Pro Trp Asn Ala Glu Lys His Ser Asn His
            1235                1240                1245

ctc gcc ttg ata ttt aag att gct agc gca act act gca ccg tcc atc   3791
Leu Ala Leu Ile Phe Lys Ile Ala Ser Ala Thr Thr Ala Pro Ser Ile
        1250                1255                1260

ccg tca cac ctg tcc cct ggt tta cga gat gtg gct ctt cgt tgt tta   3839
Pro Ser His Leu Ser Pro Gly Leu Arg Asp Val Ala Leu Arg Cys Leu
    1265                1270                1275

gaa ctt cag cct cag gac cgg cct ccg tca aga gag ctg ctg aaa cat   3887
Glu Leu Gln Pro Gln Asp Arg Pro Pro Ser Arg Glu Leu Leu Lys His
1280                1285                1290                1295
```

ccg gtc ttc cgt acc acg tgg tag 3911
Pro Val Phe Arg Thr Thr Trp
1300

<210> 6
<211> 1302
<212> PRT
<213> Homo sapiens

<400> 6
Ala Trp Lys His Glu Trp Leu Glu Arg Arg Asn Arg Arg Gly Pro Val
1 5 10 15

Val Val Lys Pro Ile Pro Val Lys Gly Asp Gly Ser Glu Met Asn His
20 25 30

Leu Ala Ala Glu Ser Pro Gly Glu Val Gln Ala Ser Ala Ala Ser Pro
35 40 45

Ala Ser Lys Gly Arg Arg Ser Pro Ser Pro Gly Asn Ser Pro Ser Gly
50 55 60

Arg Thr Val Lys Ser Glu Ser Pro Gly Val Arg Arg Lys Arg Val Ser
65 70 75 80

Pro Val Pro Phe Gln Ser Gly Arg Ile Thr Pro Pro Arg Arg Ala Pro
85 90 95

Ser Pro Asp Gly Phe Ser Pro Tyr Ser Pro Glu Glu Thr Asn Arg Arg
100 105 110

Val Asn Lys Val Met Arg Ala Arg Leu Tyr Leu Leu Gln Gln Ile Gly
115 120 125

Pro Asn Ser Phe Leu Ile Gly Gly Asp Ser Pro Asp Asn Lys Tyr Arg
130 135 140

Val Phe Ile Gly Pro Gln Asn Cys Ser Cys Ala Arg Gly Thr Phe Cys
145 150 155 160

Ile His Leu Leu Phe Val Met Leu Arg Val Phe Gln Leu Glu Pro Ser
165 170 175

Asp Pro Met Leu Trp Arg Lys Thr Leu Lys Asn Phe Glu Val Glu Ser
180 185 190

Leu Phe Gln Lys Tyr His Ser Arg Arg Ser Ser Arg Ile Lys Ala Pro
195 200 205

Ser Arg Asn Thr Ile Gln Lys Phe Val Ser Arg Met Ser Asn Ser His
210 215 220

Thr Leu Ser Ser Ser Ser Thr Ser Thr Ser Ser Ser Val Asn Ser Ile
225 230 235 240

Lys Asp Glu Glu Glu Gln Met Cys Pro Ile Cys Leu Leu Gly Met Leu
245 250 255

Asp Glu Glu Ser Leu Thr Val Cys Glu Asp Gly Cys Arg Asn Lys Leu

```
            260                 265                 270

His His His Cys Met Ser Ile Trp Ala Glu Glu Cys Arg Arg Asn Arg
        275                 280                 285

Glu Pro Leu Ile Cys Pro Leu Cys Arg Ser Lys Trp Arg Ser His Asp
    290                 295                 300

Phe Tyr Ser His Glu Leu Ser Ser Pro Val Asp Ser Pro Ser Ser Leu
305                 310                 315                 320

Arg Ala Ala Gln Gln Gln Thr Val Gln Gln Gln Pro Leu Ala Gly Ser
                325                 330                 335

Arg Arg Asn Gln Glu Ser Asn Phe Asn Leu Thr His Tyr Gly Thr Gln
            340                 345                 350

Gln Ile Pro Pro Ala Tyr Lys Asp Leu Ala Glu Pro Trp Ile Gln Val
        355                 360                 365

Phe Gly Met Glu Leu Val Gly Cys Leu Phe Ser Arg Asn Trp Asn Val
    370                 375                 380

Arg Glu Met Ala Leu Arg Arg Leu Ser His Asp Val Ser Gly Ala Leu
385                 390                 395                 400

Leu Leu Ala Asn Gly Glu Ser Thr Gly Asn Ser Gly Gly Gly Ser Gly
                405                 410                 415

Gly Ser Leu Ser Ala Gly Ala Ala Ser Gly Ser Ser Gln Pro Ser Ile
            420                 425                 430

Ser Gly Asp Val Val Glu Ala Cys Cys Ser Val Leu Ser Ile Val Cys
        435                 440                 445

Ala Asp Pro Val Tyr Lys Val Tyr Val Ala Ala Leu Lys Thr Leu Arg
    450                 455                 460

Ala Met Leu Val Tyr Thr Pro Cys His Ser Leu Ala Glu Arg Ile Lys
465                 470                 475                 480

Leu Gln Arg Leu Leu Arg Pro Val Val Asp Thr Ile Leu Val Lys Cys
                485                 490                 495

Ala Asp Ala Asn Ser Arg Thr Ser Gln Leu Ser Ile Ser Thr Val Leu
            500                 505                 510

Glu Leu Cys Asn Gly Gln Ala Gly Lys Leu Ala Val Gly Arg Glu Ile
        515                 520                 525

Leu Lys Ala Gly Ser Ile Gly Val Gly Gly Val Asp Tyr Val Leu Ser
    530                 535                 540

Cys Ile Leu Gly Asn Gln Ala Glu Ser Asn Asn Trp Gln Glu Leu Leu
545                 550                 555                 560

Gly Arg Leu Cys Leu Ile Asp Arg Leu Leu Leu Glu Phe Pro Ala Glu
                565                 570                 575

Phe Tyr Pro His Ile Val Ser Thr Asp Val Ser Gln Ala Glu Pro Val
            580                 585                 590
```

Glu Ile Arg Tyr Lys Lys Leu Leu Ser Leu Leu Thr Phe Ala Leu Gln
595 600 605

Ser Ile Asp Asn Ser His Ser Met Val Gly Lys Leu Ser Arg Arg Ile
610 615 620

Tyr Leu Ser Ser Ala Arg Met Val Thr Ala Val Pro Ala Val Phe Ser
625 630 635 640

Lys Leu Val Thr Met Leu Asn Ala Ser Gly Ser Thr His Phe Thr Arg
645 650 655

Met Arg Arg Arg Leu Met Ala Ile Ala Asp Glu Val Glu Ile Ala Glu
660 665 670

Val Ile Gln Leu Gly Val Glu Asp Thr Val Asp Gly His Gln Asp Ser
675 680 685

Leu Gln Ala Leu Ala Pro Ala Ser Cys Leu Glu Asn Ser Ser Leu Glu
690 695 700

His Thr Val His Arg Glu Lys Thr Gly Lys Gly Leu Ser Ala Thr Arg
705 710 715 720

Leu Ser Ala Ser Ser Glu Asp Ile Ser Asp Arg Leu Ala Gly Val Ser
725 730 735

Val Gly Leu Pro Ser Ser Thr Thr Thr Glu Gln Pro Lys Pro Ala Val
740 745 750

Gln Thr Lys Gly Arg Pro His Ser Gln Cys Leu Asn Ser Ser Pro Leu
755 760 765

Ser His Ala Gln Leu Met Phe Pro Ala Pro Ser Ala Pro Cys Ser Ser
770 775 780

Ala Pro Ser Val Pro Asp Ile Ser Lys His Arg Pro Gln Ala Phe Val
785 790 795 800

Pro Cys Lys Ile Pro Ser Ala Ser Pro Gln Thr Gln Arg Lys Phe Ser
805 810 815

Leu Gln Phe Gln Arg Asn Cys Ser Glu His Arg Asp Ser Asp Gln Leu
820 825 830

Ser Pro Val Phe Thr Gln Ser Arg Pro Pro Pro Ser Ser Asn Ile His
835 840 845

Arg Pro Lys Pro Ser Arg Pro Val Pro Gly Ser Thr Ser Lys Leu Gly
850 855 860

Asp Ala Thr Lys Ser Ser Met Thr Leu Asp Leu Gly Ser Ala Ser Arg
865 870 875 880

Cys Asp Asp Ser Phe Gly Gly Gly Gly Asn Ser Gly Asn Ala Val Ile
885 890 895

Pro Ser Asp Glu Thr Val Phe Thr Pro Val Glu Asp Lys Cys Arg Leu
900 905 910

Asp Val Asn Thr Glu Leu Asn Ser Ser Ile Glu Asp Leu Leu Glu Ala
915 920 925

Ser Met Pro Ser Ser Asp Thr Thr Val Thr Phe Lys Ser Glu Val Ala
930 935 940

Val Leu Ser Pro Glu Lys Ala Glu Asn Asp Asp Thr Tyr Lys Asp Asp
945 950 955 960

Val Asn His Asn Gln Lys Cys Lys Glu Lys Met Glu Ala Glu Glu Glu
965 970 975

Glu Ala Leu Ala Ile Ala Met Ala Met Ser Ala Ser Gln Asp Ala Leu
980 985 990

Pro Ile Val Pro Gln Leu Gln Val Glu Asn Gly Glu Asp Ile Ile Ile
995 1000 1005

Ile Gln Gln Asp Thr Pro Glu Thr Leu Pro Gly His Thr Lys Ala Lys
1010 1015 1020

Gln Pro Tyr Arg Glu Asp Ala Glu Trp Leu Lys Gly Gln Gln Ile Gly
025 1030 1035 1040

Leu Gly Ala Phe Ser Ser Cys Tyr Gln Ala Gln Asp Val Gly Thr Gly
1045 1050 1055

Thr Leu Met Ala Val Lys Gln Val Thr Tyr Val Arg Asn Thr Ser Ser
1060 1065 1070

Glu Gln Glu Glu Val Val Glu Ala Leu Arg Glu Glu Ile Arg Met Met
1075 1080 1085

Ser His Leu Asn His Pro Asn Ile Ile Arg Met Leu Gly Ala Thr Cys
1090 1095 1100

Glu Lys Ser Asn Tyr Asn Leu Phe Ile Glu Trp Met Ala Gly Gly Ser
105 1110 1115 1120

Val Ala His Leu Leu Ser Lys Tyr Gly Ala Phe Lys Glu Ser Val Val
1125 1130 1135

Ile Asn Tyr Thr Glu Gln Leu Leu Arg Gly Leu Ser Tyr Leu His Glu
1140 1145 1150

Asn Gln Ile Ile His Arg Asp Val Lys Gly Ala Asn Leu Leu Ile Asp
1155 1160 1165

Ser Thr Gly Gln Arg Leu Arg Ile Ala Asp Phe Gly Ala Ala Ala Arg
1170 1175 1180

Leu Ala Ser Lys Gly Thr Gly Ala Gly Glu Phe Gln Gly Gln Leu Leu
185 1190 1195 1200

Gly Thr Ile Ala Phe Met Ala Pro Glu Val Leu Arg Gly Gln Gln Tyr
1205 1210 1215

Gly Arg Ser Cys Asp Val Trp Ser Val Gly Cys Ala Ile Ile Glu Met
1220 1225 1230

Ala Cys Ala Lys Pro Pro Trp Asn Ala Glu Lys His Ser Asn His Leu

1235 1240 1245

Ala Leu Ile Phe Lys Ile Ala Ser Ala Thr Thr Ala Pro Ser Ile Pro
1250 1255 1260

Ser His Leu Ser Pro Gly Leu Arg Asp Val Ala Leu Arg Cys Leu Glu
265 1270 1275 1280

Leu Gln Pro Gln Asp Arg Pro Pro Ser Arg Glu Leu Leu Lys His Pro
1285 1290 1295

Val Phe Arg Thr Thr Trp
1300

<210> 7
<211> 4
<212> PRT
<213> Murine

<400> 7
Asp Glu Val Glu
1

<210> 8
<211> 4
<212> PRT
<213> Murine

<400> 8
Asp Thr Val Asp
1

<210> 9
<211> 15
<212> PRT
<213> Synthetic Construct

<400> 9
Asp Arg Pro Pro Ser Arg Glu Leu Leu Lys His Pro Val Glu Arg
1 5 10 15

<210> 10
<211> 4
<212> PRT
<213> Synthetic Construct

<400> 10
Tyr Val Ala Asp
1

<210> 11
<211> 4
<212> PRT
<213> Synthetic Construct

<400> 11

Asp Glu Val Asp
1

<210> 12
<211> 11
<212> PRT
<213> Synthetic Construct

<400> 12
Met Gly Tyr Pro Tyr Asp Val Asp Tyr Ala Ser
1 5 10

<210> 13
<211> 20
<212> DNA
<213> Mus Musculus

<400> 13
gaacaccatc cagaagtttg 20

<210> 14
<211> 21
<212> DNA
<213> Mus Musculus

<400> 14
cactttgtag acagggtcag c 21

<210> 15
<211> 25
<212> DNA
<213> Homo Sapiens

<400> 15
tgggtcgcct ctgtcttata gacag 25

<210> 16
<211> 20
<212> DNA
<213> Homo sapiens

<400> 16
cacatcctgt gcttggtaac 20

<210> 17
<211> 21
<212> DNA
<213> Homo sapiens

<400> 17
aggacaagtg caggttagat g 21

<210> 18
<211> 21

<212> DNA
<213> Homo sapiens

<400> 18
gctgtccata tctacagtgc t 21

<210> 19
<211> 21
<212> DNA
<213> Homo sapiens

<400> 19
cggcctggaa gcacgagtgg t 21

<210> 20
<211> 21
<212> DNA
<213> Homo sapiens

<400> 20
ttcatccttg atgctgtttt c 21

<210> 21
<211> 1493
<212> PRT
<213> Rattus Species

<400> 21

```
Met Ala Ala Ala Ala Gly Asp Arg Ala Ser Ser Ser Gly Phe Pro Gly
  1               5                  10                  15

Ala Ala Ala Ala Ser Pro Glu Ala Gly Gly Gly Gly Gly Ala Leu Gln
             20                  25                  30

Gly Ser Gly Ala Pro Ala Ala Gly Ala Gly Leu Leu Arg Glu Thr Gly
         35                  40                  45

Ser Ala Gly Arg Glu Arg Ala Asp Trp Arg Arg Gln Gln Leu Arg Lys
     50                  55                  60

Val Arg Ser Val Glu Leu Asp Gln Leu Pro Glu Gln Pro Leu Phe Leu
 65                  70                  75                  80

Thr Ala Ser Pro Pro Cys Pro Ser Thr Ser Pro Ser Pro Glu Pro Ala
                 85                  90                  95

Asp Ala Ala Ala Gly Ala Ser Gly Phe Gln Pro Ala Ala Gly Pro Pro
            100                 105                 110

Pro Pro Gly Ala Ala Ser Arg Cys Gly Ser His Ser Ala Glu Leu Ala
        115                 120                 125

Ala Ala Arg Asp Ser Gly Ala Arg Ser Pro Ala Gly Ala Glu Pro Pro
    130                 135                 140

Ser Ala Ala Ala Pro Ser Gly Arg Glu Met Glu Asn Lys Glu Thr Leu
145                 150                 155                 160
```

Lys Gly Leu His Lys Met Asp Asp Arg Pro Glu Glu Arg Met Ile Arg
165 170 175

Glu Lys Leu Lys Ala Thr Cys Met Pro Ala Trp Lys His Glu Trp Leu
180 185 190

Glu Arg Arg Asn Arg Arg Gly Pro Val Val Val Lys Pro Ile Pro Ile
195 200 205

Lys Gly Asp Gly Ser Glu Met Ser Asn Leu Ala Ala Glu Leu Gln Gly
210 215 220

Glu Gly Gln Ala Gly Ser Ala Ala Pro Ala Pro Lys Gly Arg Arg Ser
225 230 235 240

Pro Ser Pro Gly Ser Ser Pro Ser Gly Arg Ser Gly Lys Pro Glu Ser
245 250 255

Pro Gly Val Arg Arg Lys Arg Val Ser Pro Val Pro Phe Gln Ser Gly
260 265 270

Arg Ile Thr Pro Pro Arg Arg Ala Pro Ser Pro Asp Gly Phe Ser Pro
275 280 285

Tyr Ser Pro Glu Glu Thr Ser Arg Arg Val Asn Lys Val Met Arg Ala
290 295 300

Arg Leu Tyr Leu Leu Gln Gln Ile Gly Pro Asn Ser Phe Leu Ile Gly
305 310 315 320

Gly Asp Ser Pro Asp Asn Lys Tyr Arg Val Phe Ile Gly Pro Gln Asn
325 330 335

Cys Ser Cys Gly Arg Gly Thr Phe Cys Ile His Leu Leu Phe Val Met
340 345 350

Leu Arg Val Phe Gln Leu Glu Pro Ser Asp Pro Met Leu Trp Arg Lys
355 360 365

Thr Leu Lys Asn Phe Glu Val Glu Ser Leu Phe Gln Lys Tyr His Ser
370 375 380

Arg Arg Ser Ser Arg Ile Lys Ala Pro Ser Arg Asn Thr Ile Gln Lys
385 390 395 400

Phe Val Ser Arg Met Ser Asn Cys His Thr Leu Ser Ser Ser Ser Thr
405 410 415

Ser Thr Ser Ser Ser Glu Asn Ser Ile Lys Asp Glu Glu Glu Gln Met
420 425 430

Cys Pro Ile Cys Leu Leu Gly Met Leu Asp Glu Glu Ser Leu Thr Val
435 440 445

Cys Glu Asp Gly Cys Arg Asn Lys Leu His His His Cys Met Ser Ile
450 455 460

Trp Ala Glu Glu Cys Arg Arg Asn Arg Glu Pro Leu Ile Cys Pro Leu
465 470 475 480

Cys Arg Ser Lys Trp Arg Ser His Asp Phe Tyr Ser His Glu Leu Ser

485 490 495

Ser Pro Val Asp Ser Pro Thr Ser Leu Arg Gly Val Gln Gln Pro Ser
500 505 510

Ser Pro Gln Gln Pro Val Ala Gly Ser Gln Arg Arg Asn Gln Glu Ser
515 520 525

Asn Phe Asn Leu Thr His Tyr Gly Thr Gln Gln Ile Pro Pro Ala Tyr
530 535 540

Lys Asp Leu Ala Glu Pro Trp Ile Gln Ala Phe Gly Met Glu Leu Val
545 550 555 560

Gly Cys Leu Phe Ser Arg Asn Trp Asn Val Arg Glu Met Ala Leu Arg
565 570 575

Arg Leu Ser His Asp Val Ser Gly Ala Leu Leu Leu Ala Asn Gly Glu
580 585 590

Ser Thr Gly Thr Ser Gly Gly Gly Ser Gly Gly Ser Leu Ser Ala Gly
595 600 605

Ala Ala Ser Gly Ser Ser Gln Pro Ser Ile Ser Gly Asp Val Val Glu
610 615 620

Ala Phe Cys Ser Val Leu Ser Ile Val Cys Ala Asp Pro Val Tyr Lys
625 630 635 640

Val Tyr Val Ala Ala Leu Lys Thr Leu Arg Ala Met Leu Val Tyr Thr
645 650 655

Pro Cys His Ser Leu Ala Glu Arg Ile Lys Leu Gln Arg Leu Leu Arg
660 665 670

Pro Val Val Asp Thr Ile Leu Val Lys Cys Ala Asp Ala Asn Ser Arg
675 680 685

Thr Ser Gln Leu Ser Ile Ser Thr Leu Leu Glu Leu Cys Lys Gly Gln
690 695 700

Ala Gly Glu Leu Ala Val Gly Arg Glu Ile Leu Lys Ala Gly Ser Ile
705 710 715 720

Gly Val Gly Gly Val Asp Tyr Val Leu Ser Cys Ile Leu Gly Asn Gln
725 730 735

Ala Glu Ser Asn Asn Trp Gln Glu Leu Leu Gly Arg Leu Cys Leu Ile
740 745 750

Asp Arg Leu Leu Leu Glu Ile Ser Ala Glu Phe Tyr Pro His Ile Val
755 760 765

Ser Thr Asp Val Ser Gln Ala Glu Pro Val Glu Ile Arg Tyr Lys Lys
770 775 780

Leu Leu Ser Leu Leu Ala Phe Ala Leu Gln Ser Ile Asp Asn Ser His
785 790 795 800

Ser Met Val Gly Lys Leu Ser Arg Arg Ile Tyr Leu Ser Ser Ala Arg
805 810 815

Met Val Thr Thr Val Pro Pro Leu Phe Ser Lys Leu Val Thr Met Leu
820 825 830

Ser Ala Ser Gly Ser Ser His Phe Ala Arg Met Arg Arg Arg Leu Met
835 840 845

Ala Ile Ala Asp Glu Val Glu Ile Ala Glu Val Ile Gln Leu Gly Ser
850 855 860

Glu Asp Thr Leu Asp Gly Gln Gln Asp Ser Ser Gln Ala Leu Ala Pro
865 870 875 880

Pro Arg Tyr Pro Glu Ser Ser Ser Leu Glu His Thr Ala His Val Glu
885 890 895

Lys Thr Gly Lys Gly Leu Lys Ala Thr Arg Leu Ser Ala Ser Ser Glu
900 905 910

Asp Ile Ser Asp Arg Leu Ala Gly Val Ser Val Gly Leu Pro Ser Ser
915 920 925

Ala Thr Thr Glu Gln Pro Lys Pro Thr Val Gln Thr Lys Gly Arg Pro
930 935 940

His Ser Gln Cys Leu Asn Ser Ser Pro Leu Ser Pro Pro Gln Leu Met
945 950 955 960

Phe Pro Ala Ile Ser Ala Pro Cys Ser Ser Ala Pro Ser Val Pro Ala
965 970 975

Gly Ser Val Thr Asp Ala Ser Lys His Arg Pro Arg Ala Phe Val Pro
980 985 990

Cys Lys Ile Pro Ser Ala Ser Pro Gln Thr Gln Arg Lys Phe Ser Leu
995 1000 1005

Gln Phe Gln Arg Thr Cys Ser Glu Asn Arg Asp Ser Glu Lys Leu Ser
1010 1015 1020

Pro Val Phe Thr Gln Ser Arg Pro Pro Pro Ser Ser Asn Ile His Arg
1025 1030 1035 1040

Ala Lys Ala Ser Arg Pro Val Pro Gly Ser Thr Ser Lys Leu Gly Asp
1045 1050 1055

Ala Ser Lys Asn Ser Met Thr Leu Asp Leu Asn Ser Ala Ser Gln Cys
1060 1065 1070

Asp Asp Ser Phe Gly Ser Gly Ser Asn Ser Gly Ser Ala Val Ile Pro
1075 1080 1085

Ser Glu Glu Thr Ala Phe Thr Pro Ala Glu Asp Lys Cys Arg Leu Asp
1090 1095 1100

Val Asn Pro Glu Leu Asn Ser Ser Ile Glu Asp Leu Leu Glu Ala Ser
1105 1110 1115 1120

Met Pro Ser Ser Asp Thr Thr Val Thr Phe Lys Ser Glu Val Ala Val
1125 1130 1135

Leu Ser Pro Glu Lys Ala Glu Ser Asp Asp Thr Tyr Lys Asp Asp Val
1140 1145 1150

Asn His Asn Gln Lys Cys Lys Glu Lys Met Glu Ala Glu Glu Glu Glu
1155 1160 1165

Ala Leu Ala Ile Ala Met Ala Met Ser Ala Ser Gln Asp Ala Leu Pro
1170 1175 1180

Ile Val Pro Gln Leu Gln Val Glu Asn Gly Glu Asp Ile Ile Ile Ile
1185 1190 1195 1200

Gln Ala Asp Thr Pro Glu Thr Leu Pro Gly His Thr Lys Ala Asn Glu
1205 1210 1215

Pro Tyr Arg Glu Asp Thr Glu Trp Leu Lys Gly Gln Gln Ile Gly Leu
1220 1225 1230

Gly Ala Phe Ser Ser Cys Tyr Gln Ala Gln Asp Val Gly Thr Gly Thr
1235 1240 1245

Leu Met Ala Val Lys Gln Val Thr Tyr Val Arg Asn Thr Ser Ser Glu
1250 1255 1260

Gln Glu Glu Val Val Glu Ala Leu Arg Glu Glu Ile Arg Met Met Ser
1265 1270 1275 1280

His Leu Asn His Pro Asn Ile Ile Arg Met Leu Gly Ala Thr Cys Glu
1285 1290 1295

Lys Ser Asn Tyr Asn Leu Phe Ile Glu Trp Met Ala Gly Ala Ser Val
1300 1305 1310

Ala His Leu Leu Ser Lys Tyr Gly Ala Phe Lys Glu Ser Val Val Ile
1315 1320 1325

Asn Tyr Thr Glu Gln Leu Leu Arg Gly Leu Ser Tyr Leu His Glu Asn
1330 1335 1340

Gln Ile Ile His Arg Asp Val Lys Gly Ala Asn Leu Leu Ile Asp Ser
1345 1350 1355 1360

Thr Gly Gln Arg Leu Arg Ile Ala Asp Phe Gly Ala Ala Ala Arg Leu
1365 1370 1375

Ala Ser Lys Gly Thr Gly Ala Gly Glu Phe Gln Gly Gln Leu Leu Gly
1380 1385 1390

Thr Ile Ala Phe Met Ala Pro Glu Val Leu Arg Gly Gln Gln Tyr Gly
1395 1400 1405

Arg Ser Cys Asp Val Trp Ser Val Gly Cys Ala Ile Ile Glu Met Ala
1410 1415 1420

Cys Ala Lys Pro Pro Trp Asn Ala Glu Lys His Ser Asn His Leu Ala
1425 1430 1435 1440

Leu Ile Phe Lys Ile Ala Ser Ala Thr Thr Ala Pro Ser Ile Pro Ser
1445 1450 1455

His Leu Ser Pro Gly Leu Arg Asp Val Ala Leu Arg Cys Leu Glu Leu

1460 1465 1470

Gln Pro Gln Asp Arg Pro Pro Ser Arg Glu Leu Leu Lys His Pro Val
1475 1480 1485

Phe Arg Thr Thr Trp
1490

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
CPI-042CPPC		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/ 02974	12/02/1999	13/02/1998
Applicant		
CADUS PHARMACEUTICAL CORPORATION et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[X] furnished subsequently to this Authority in computer readble form.

[X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 6

[ ] as suggested by the applicant.

[X] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/02974

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/54 C12N9/12 C12N5/10 C12N15/62 C07K19/00
C07K16/40 G01N33/573 C12Q1/68 A61K31/00 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K G01N C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WIDMANN C. ET AL.: "Potentiation of apoptosis by low dose stress stimuli in cells expressing activated mEK kinase 1." ONCOGENE, vol. 15, no. 20, November 1997 (1997-11), pages 2439-2447, XP002108366 the whole document ---	1-67
X	FANGER G. R. ET AL.: "MEK kinases are regulated by EGF and selectively interact with Rac/Cdc42." EMBO JOURNAL, vol. 16, no. 16, 1997, pages 4961-4972, XP002108750 figure 1 page 4969, right-hand column, paragraphs 2,3 --- -/--	1-18,21, 26-33, 43-51, 58,60

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 July 1999	22/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mandl, B

3

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 28421 A (NAT JEWISH CENTER FOR IMMUNOLO ;JOHNSON GARY L (US)) 26 October 1995 (1995-10-26) the whole document	1-33, 43-51, 58,60, 63,66
X	XU S. ET AL.: "Cloning of rat MEK kinase 1 cDNA reveals an endogenous membrane-associated 195 kDa protein with a large regulatory domain." PROC. NATL. ACAD. SCI. USA, vol. 93, May 1996 (1996-05), pages 5291-5295, XP002108363 cited in the application the whole document	1,2,4-6, 8,9, 11-13, 15-21, 26-33, 43-51, 58,60
X	LANGE-CARTER C. A. ET AL.: "A divergence in the MAP kinase regulatory network defined by MEK kinase and Raf." SCIENCE, vol. 260, 16 April 1993 (1993-04-16), pages 315-319, XP002108364 cited in the application the whole document	1,2,4-6, 8,9, 11-13, 15-21, 26-33, 43-51, 58,60
X	CARDONE M. H. ET AL.: "The regulation of Anoikis: MEKK-1 activation requires cleavage by caspases." CELL, vol. 90, 25 July 1997 (1997-07-25), pages 315-323, XP002108365	34, 37-42, 52-57, 59,61, 62,65,67
Y	the whole document	35,36
Y	WO 97 40145 A (UNIV MICHIGAN ;KAUFMAN RANDAL J (US); PIPE STEVEN W (US); AMANO KA) 30 October 1997 (1997-10-30) page 6, line 1 - line 2	35,36
X	EMBL database entry AF042838; accession number AF042838; 31-Jan-98; Xia Y. et al.: 'Homo sapiens MEK kinase 1 (MEKK1) mRNA, partial codings.' XP002108368 abstract	1,5
P,X	WIDMANN C. ET AL.: "MEK kinase 1, a substrate for DEVD-directed caspases, is involved in genotoxin-induced apoptosis." MOL. CELL. BIOL., vol. 18, no. 4, April 1998 (1998-04), pages 2416-2429, XP002108367 the whole document	1-67

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	XIA Y. ET AL.: "JNKK1 organizes a MAP kinase module through specific and sequential interactions with the upstream and downstream components mediated by its amino-terminal extension." GENES AND DEVELOPMENT, vol. 12, no. 21, 1 November 1998 (1998-11-01), pages 3369-3381, XP002108751 page 3370, right-hand column, paragraph 2 - page 3371, left-hand column, paragraph 1 -----	1,2,4-6, 8,9,12, 13,16, 26-33, 43-51

INTERNATIONAL SEARCH REPORT

[redacted]ional application No.
PCT/US 99/02974

Box I Obs rvations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
See FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claim 24 and claims 22, 23, 25, 63 and 64, as far as an in vivo application is concerned, are directed to a method of treatment of the human/animal body or to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/02974

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9528421 A	26-10-1995	US 5405941 A	11-04-1995
		AU 697340 B	01-10-1998
		AU 6666394 A	08-11-1994
		CA 2160548 A	27-10-1994
		EP 0694044 A	31-01-1996
		EP 0755406 A	29-01-1997
		FI 954894 A	13-10-1995
		JP 8509128 T	01-10-1996
		NO 954094 A	13-12-1995
		NZ 266067 A	22-09-1997
		PL 311142 A	05-02-1996
		WO 9424159 A	27-10-1994
		US 5854043 A	29-12-1998
		US 5753446 A	19-05-1998
		AU 703070 B	11-03-1999
		AU 8017794 A	10-11-1995
		CA 2186526 A	26-10-1995
WO 9740145 A	30-10-1997	AU 3202797 A	12-11-1997
		EP 0910628 A	28-04-1999